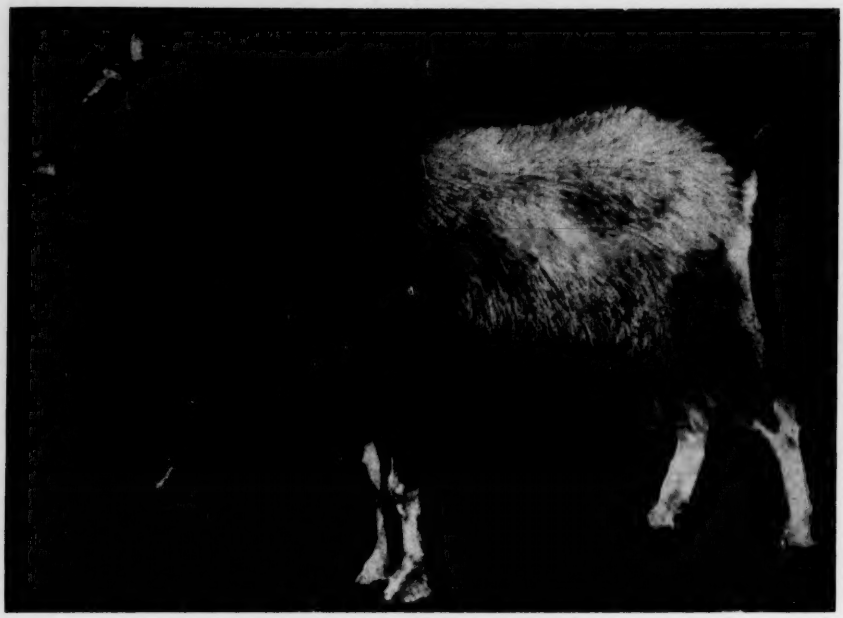
The Journal of HEREDITY



CAPRINE BRITISH ARISTOCRAT

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Furless Mice

Chestnut Breeding

Chromosomal Banding Patterns

Retinal Degeneration in the Mouse

Electron Microscopy of Salivary Chromosomes

VOLUME 45 MAY-JUNE, 1954 NUMBER 3

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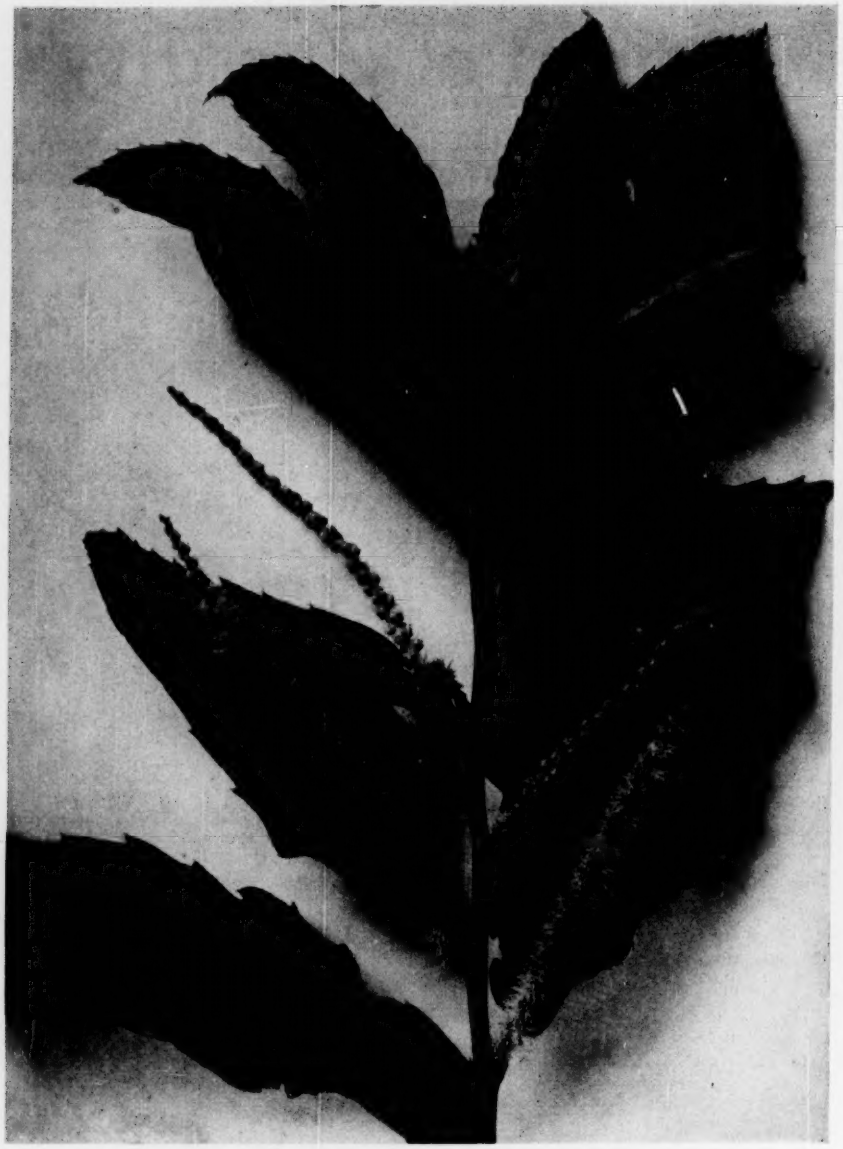


INFLORESCENCE OF THE CHINESE CHESTNUT

Figure 1

The chestnut is monoecious, with staminate and pistillate catkins on the same tree. Both kinds of catkins produce pollen, but the staminate catkins begin to shed pollen about 10 days before the pistillate catkins. The chestnut is usually self-sterile.

CHESTNUT BREEDING, TECHNIQUES AND RESULTS*

I. Breeding Material and Pollination Techniques

RUSSELL B. CLAPPER†

THE American chestnut was formerly an important forest timber tree in the states east of the Mississippi River. Its wood was durable and an important source of tannin used in the tanning of heavy leathers. In 1904, American chestnut trees in New York City were found dying from a blight, later determined to be caused by the fungus *Endothia parasitica*. This fungus had unknowingly been brought to this country some years before on infected chestnut nursery stock from Asia. Attempts to control the blight made it possible to utilize chestnut timber in some areas before the trees were attacked but within four decades, the blight had almost eliminated the American chestnut.

The task of breeding chestnut trees resistant to the blight began in the Division of Forest Pathology in 1909 [12, 13]. The purpose of the breeding work was to develop blight-resistant chestnut trees to replace the American chestnut. The objectives at first were twofold: (1) the development of a blight-resistant forest chestnut for tannin, timber, and food for wildlife, and (2) the development of blight-resistant selections producing nuts of high quality for orchard planting. About 1930 most of the orchard chestnut work was transferred to the Division of Fruit and Nut Crops.

Breeding Material and Techniques

Species of *Castanea*

The American chestnut (*Castanea dentata* (Marsh.) Borkh.) was very susceptible to the blight fungus. The fungus was unable to kill parts of the tree below ground, and many dying chestnut trees produced basal sprouts. These sprouts would grow for a number of years, become blighted, die, and new sprouts would take their place. They are now the principal form of the American chestnut with which the breeder works. No completely blight-resistant mature American chestnut has been found. The species, even though it is susceptible to the blight, is valuable to the breeder because it produces larger forest trees than any other chestnut species. The American chestnut is an important constituent in crosses made to develop a fast-growing hybrid for timber.

The Chinese chestnut (*C. mollissima* Blume), though a smaller tree than our native chestnut, is sufficiently resistant to the blight to justify extensive plantings in orchards. Seedlings of a few introductions have been established in numerous, small-scale forest plantings. The nuts vary in size, from those of the native American chestnut to those of the European chestnut seen in the markets in the fall. The flavor is usually sweet, but the nut lacks the aroma of the na-

*EDITOR'S NOTE: Since this report is necessarily of considerable length, it will be printed in two consecutive issues. Literature citations referred to in Part I will appear in their entirety at the conclusion of the article.

†Formerly Associate Pathologist, now Collaborator, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture.

Walter Van Fleet conducted the breeding work from 1909 to 1922. The writer worked on this project from 1925 to 1949. Frederick H. Berry now conducts this work. G. F. Gravatt has general charge of the chestnut project in the Division of Forest Pathology. Arthur H. Graves, formerly of the Brooklyn Botanic Garden, began breeding chestnuts in 1930, to develop a blight-resistant timber tree. The Division of Forest Pathology has co-operated in this work by supplying some of the breeding stock and funds. The work is being continued by Dr. Graves and Dr. Hans Nienstaedt under the auspices of the Connecticut Agricultural Experiment Station.

tive chestnut. The Division of Forest Pathology imported, for propagation, large quantities of scions and especially nuts of the Chinese chestnut from 1933 to 1936. This species, unlike the American, contains morphological variants differing in size and color of nuts and size, shape, and serration of leaves. The Chinese chestnut is the most important constituent in crosses made to obtain new, blight-resistant timber trees.

The Japanese chestnut tree (*C. crenata* Sieb. & Zucc.) attains about the same size as the Chinese but appears to be less resistant to the blight. Both the wild forms and cultivated varieties produce large nuts, but their flavor is usually not pleasing. Both forms have been crossed with the American chestnut, but the hybrids were not promising for forest plantings. Selections of the Japanese and Chinese chestnuts have also been crossed to produce fully blight-resistant hybrids, but in most instances poor tree form or slow rate of growth eliminated them from use in forest plantings.

The Henry chinkapin (*C. henryi* (Skan) Rehd. & Wils.) grows as a timber tree in China, but blight and winter injuries in this country usually retard and deform the trees. Hybrids between *C. henryi* and *C. mollissima* were severely injured by freezes at Glenn Dale, Md. Hybrids between *C. henryi* and *C. ozarkensis*, the Ozark tree chinkapin, are low, branchy trees. In recent years the Henry chinkapin was crossed with the American chestnut; the hybrids are expected to show good forest-tree form, but susceptibility to the blight will eliminate their use as first-generation hybrids.

The Seguin chestnut (*C. seguinii* Dode) of China grows there as a small tree, but in this country it usually is a bush or shrubby tree. It is usually everbearing, with the nuts ripening progressively outward on the branches for a period of several weeks. The Seguin chestnut is resistant to the blight but susceptible to early fall freezes that cause dieback. Attempts are being made to transfer the productiveness of the Seguin chestnut to more hardy hybrids for use in wildlife plantings.

The European chestnut (*C. sativa* Mill.) and some of its varieties were formerly grown in orchards in this country, but the blight eliminated them. They are also susceptible to root rot fungi and winter injury. This species has very few characteristics important to the chestnut breeder, and has not been used much in American breeding work.

Several species of chinkapins are native to southern and southeastern states: *C. pumila* (L.) Mill., *C. ashei* (Sudw.), × *C. alabamensis* Ashe, *C. ozarkensis* Ashe, *C. alnifolia* var. *floridana* Sarg., and *C. paucispina* Ashe. These species range in growth habit from short stemmed as *C. alnifolia* and *C. paucispina*, to large-tree type, as *C. ozarkensis*. All the chinkapins are susceptible to the blight, but their ability to sprout multiple stems aids in their survival.

Trees of the foregoing species of chestnuts and most of the chinkapins are growing in the Federal test plots near Glenn Dale, Md., and have been used in various combinations of crosses. The success with which fertile hybrids were obtained from these crosses suggests that all the species of *Castanea* have the same number of chromosomes, 24. Some 40 percent of the total possible number of combinations of crosses have been made successfully (Table I), indicating the close relationship existing between the species of *Castanea*.

In the period 1926-49, a total of 18,511 nuts were obtained from crossings. The nuts from each year's work were packed in moist moss or sawdust and stored at 33° to 38° F. for about three months. The seed was then planted in a warm greenhouse. Records of the percentage of germination were kept for 10 years, on a total of 10,512 nuts. The weighted average percentage of germination was 59 percent.

Controlling Pollination

All species of *Castanea* are monoecious, with male and female flowers separate on the same plant. The male flowers form staminate

catkins, and the female flowers appear at the bases of otherwise staminate catkins that usually grow near the tip of the branch (Figure 1).

Staminate and pistillate flowers are enclosed in separate bags, so that the pollen will not be lost or contaminated, and the pistils will not be pollinated by wind or insects. Kraft paper bags with square bottoms, sizes 8 and 10, are preferred. Heat is not excessive with-

TABLE I. ***Castanea*** **hybrids produced from 1926 to 1949**

Crosses[1]	No. of Plants[2]
Boone[3] × *crenata*	22
Boone × *crenata*-w[4]	1
Boone × *mollissima*	12
(Boone × *mollissima*) × (*crenata*-w × *floridana*)	6
(Boone × *mollissima*) × *mollissima*	13
xchinkapin[6] × xchinkapin	24
xchinkapin × *xseguinii*	11
crenata × *alabamensis*	13
crenata × chinkapin	1
crenata × xchinkapin	2
crenata × *crenata*	195
crenata × *dentata*	53
(*crenata* × *dentata*) × *crenata*	39
(*crenata* × *dentata*) × (*crenata* × *dentata*)	25
(*crenata* × *dentata*) × (*crenata* × *mollissima*)	2
(*crenata* × *dentata*) × *mollissima*	25
(*crenata* × *dentata*) × (*mollissima* × *dentata*)	16
(*crenata* × *dentata*) × *ozarkensis*	5
(*crenata* × *dentata*) × *sativa*	5
crenata × *mollissima*	194
(*crenata* × *mollissima*) × *mollissima*	22
(*crenata* × *mollissima*) × (*crenata* × *mollissima*)	162
(*crenata* × *mollissima*) F_2 × *mollissima*	4
(*crenata* × *mollissima*) × (*mollissima* × *dentata*)	21
crenata × *ozarkensis*	8
crenata × *pumila*	47
(*crenata* × *pumila*) × *crenata*	37
(*crenata* × *pumila*) × (*crenata* × *pumila*)	2
crenata × *sativa*	13
crenata × *seguinii*	8
(*crenata* × *seguinii*) × (*crenata* × *seguinii*)	12
crenata × *xseguinii*	8
(*crenata* × *xseguinii*) × (*crenata* × *xseguinii*	8
crenata-s[5] × *crenata*	5
crenata-s × *crenata*-w	29
crenata-s × *mollissima*	7
crenata-s × *pumila*	10
crenata-w × *alabamensis*	22
(*crenata*-w × *alabamensis*) × *crenata*	1
(*crenata*-w × *alabamensis*) × *mollissima*	3
crenata-w × *alnifolia*	4
crenata-w × chinkapin	4
crenata-w × *crenata*-w	1
crenata-w × *dentata*	23
(*crenata*-w × *dentata*) × *crenata*	37
(*crenata*-w × *dentata*) × *crenata*-w	5
(*crenata*-w × *dentata*) × (*crenata*-w × *dentata*)	3
(*crenata*-w × *dentata*) × *dentata*	1
(*crenata*-w × *dentata*) × *mollissima*	11
(*crenata*-w × *dentata*) × (*mollissima* × *dentata*)	1
crenata-w × *floridana*	8
(*crenata*-w × *floridana*) × (*crenata*-w × *floridana*)	7
crenata-w × *henryi*	2
crenata-w × *alnifolia* var. *floridana*	9
(*crenata*-w × *alnifolia* var. *floridana*) × (*crenata*-w × *alnifolia* var. *floridana*)	10
crenata-w × *mollissima*	51
(*crenata*-w × *mollissima*) × (*mollissima* × *dentata*)	11
crenata-w × *ozarkensis*	12
crenata-w × *pumila*	6
crenata-w × *seguinii*	3
dentata × chinkapin	9
dentata × *floridana*	8
(*dentata* × *floridana*) × (*dentata* × *floridana*)	1
(*dentata* × *floridana*) × *mollissima*	25
dentata × *henryi*	5
dentata × *pumila*	3
dentata × *seguinii*	13
dentata × *xseguinii*	17
(*dentata* × *seguinii*) × *dentata*	2
(*dentata* × *seguinii*) × *mollissima*	1
(*dentata* × *seguinii*) × *seguinii*	3
Fuller[7] × *mollissima*	4
(Fuller × *mollissima*) × *crenata*	4
henryi × *ozarkensis*	13
henryi × *pumila*	10
(*henryi* × *pumila*) × (*henryi* × *pumila*)	2
henryi × *seguinii*	6
Miracle[9] × *mollissima*	4
Miracle × *seguinii*	1
mollissima × *alabamensis*	6
mollissima × *alnifolia*	7
mollissima × *athei*	7
mollissima × chinkapin	5
mollissima × xchinkapin	47
mollissima × *dentata*	388
(*mollissima* × *dentata*) × *crenata*	87
(*mollissima* × *dentata*) × *crenata*-w	14
(*mollissima* × *dentata*) × *dentata*	37
(*mollissima* × *dentata*) × *henryi*	16
(*mollissima* × *dentata*) × *mollissima*	405
(*mollissima* × *dentata*) × (*mollissima* × *dentata*)	200
(*mollissima* × *dentata*) × (*mollissima* × *mollissima*)	13
((*mollissima* × *dentata*) × *mollissima*)) × *mollissima*	74
mollissima × *floridana*	4
mollissima × *henryi*	33
mollissima × *alnifolia* var. *floridana*	1
mollissima × *mollissima*	632
(*mollissima* × *mollissima*) × *mollissima*	8
(*mollissima* × *mollissima*) × (*mollissima* × *mollissima*)	12
mollissima × *ozarkensis*	15
mollissima × *paucispina*	1
mollissima × *pumila*	46
(*mollissima* × *pumila*) × *mollissima*	12
(*mollissima* × *pumila*) × (*mollissima* × *pumila*)	10
mollissima × *sativa*	2
mollissima × *seguinii*	82
(*mollissima* × *seguinii*) × *mollissima*	1
(*mollissima* × *seguinii*) × (*mollissima* × *seguinii*)	73
mollissima × *xseguinii*	56
(*mollissima* × *xseguinii*) × *xseguinii*	45
(*mollissima* × *xseguinii*) × (*mollissima* × *xseguinii*)	14
016[8] × chinkapin	3
016 × xchinkapin	18
016 × *crenata*	227
(016 × *crenata*) × (016 × *crenata*)	11
(016 × *crenata*) × (*crenata* × *dentata*)	5
(016 × *crenata*) × *mollissima*	12
(016 × *crenata*) × 016	40
016 × *dentata*	39
(016 × *dentata*) × 016	32
(016 × *dentata*) × (016 × *dentata*)	3
016 × *mollissima*	269
(016 × *mollissima*) × 016	1
(016 × *mollissima*) × (016 × *mollissima*)	15
016 × (*mollissima* × *dentata*)	4
016 × *ozarkensis*	3
016 × *pumila*	3
016 × S8[10]	71
016 × *seguinii*	13
016 × *xseguinii*	19
(016 × *xseguinii*) × (016 × *xseguinii*)	42
Paragon[7] × *crenata*	15
Paragon × *mollissima*	18
RG1[8] × xchinkapin	30
RG1 × *crenata*	94
RG1 × *mollissima*	79
RG1 × (*mollissima* × *mollissima*)	23
(RG1 × *mollissima*) × *mollissima*	11
(RG1 × *mollissima*) × RG1	2
(RG1 × *mollissima*) × (RG1 × *mollissima*)	4
RG1 × S8	110
S8 × chinkapin	33
S8 × xchinkapin	8
S8 × *crenata*	397
(S8 × *crenata*) × *crenata*	51
(S8 × *crenata*) × S8	132
(S8 × *crenata*) × (S8 × *crenata*)	62
S8 × *dentata*	47
(S8 × *dentata*) × S8	21
(S8 × *dentata*) × *mollissima*	1
S8 × *henryi*	1
S8 × *mollissima*	197
(S8 × *mollissima*) × *mollissima*	18
(S8 × *mollissima*) × S8	96
(S8 × *mollissima*) × (S8 × *mollissima*)	98
(S8 × *mollissima*) F_2 × (S8 × *mollissima*) F_2	2
S8 × (*mollissima* × *xseguinii*)	4
S8 × *pumila*	17
(S8 × *pumila*) × *crenata*	35
(S8 × *pumila*) × S8	45
S8 × *seguinii*	5
(S8 × *seguinii*) × S8	20
S8 × *xseguinii*	13
(S8 × *xseguinii*) × (S8 × *xseguinii*)	8
seguinii × *alabamensis*	7
seguinii × *alnifolia*	3
seguinii × *floridana*	2
seguinii × *ozarkensis*	1
seguinii × *pumila*	20
(*seguinii* × *pumila*) × *crenata*	1
(*seguinii* × *pumila*) × *seguinii*	3
(*seguinii* × *pumila*) × (*seguinii* × *pumila*)	14
seguinii × *xseguinii*	5

[1] Reciprocal crosses are not listed as such, so the parent given first is not necessarily the female.
[2] The number of plants transplanted from the greenhouse to the test garden.
[3] Seedlings of *C. crenata* × *C. dentata*, a cross made by Endicott.
[4] The wild form of *C. crenata*.
[5] A spineless-burr mutant.
[6] "x" before a name indicates an unknown hybrid.
[7] A variety of *C. sativa*.
[8] A *C. mollissima* × *C. crenata* hybrid of Van Fleet.
[9] A chinkapin hybrid of Burbank's.
[10] A chinkapin hybrid of Van Fleet.

in the bags and each bag can hold several flowering shoots. Rain may open the glued seams of the paper bag unless it is reinforced by brushing a clear lacquer or cement over it before using. Kraft bags with waterproofed seams are also available.

When female flowers are prepared for bagging, all staminate catkins and usually the staminate parts of the pistillate catkins are pinched off. This emasculation is done despite the fact that chestnut and chinkapin are usually self-sterile. The female flowers are bagged before their styles begin to bend over. A bag is placed over the branch bearing the female flowers; the mouth of the bag is wrapped tightly around the stem of the branch and secured with a wired tree label.

Staminate catkins are bagged a few days before they begin to blossom. Pistillate catkins, if present, may be included with the staminate in the bag, since the staminate parts of the former can furnish pollen for late pollinations. Also, if the involucres develop into fertile burrs, it may indicate that the tree is capable of setting seed by its own pollen. When bagging either male or female flowers, it may be desirable to pinch off some of the leaves, to facilitate operations.

Bags containing mature staminate catkins are handled carefully so that the pollen will not be dislodged. When hand pollinations are made, several mature catkins are carefully removed from a bag and placed in a small metal container. A bag enclosing female flowers is removed from the mother tree, and one or more of the catkins are brushed over the styles of the female flowers (Figure 2). After pollination the bag is replaced.

Female flowers are not pollinated until they are fully receptive. They are receptive when the styles have turned from a green color to straw-yellow, and have spread more or less at right angles to the axis of the flower. Another method of determining the receptive period is to note when staminate catkins on the mother tree begin to blossom; about 12 days after that date the stigmas will be receptive.

The female flowers remain bagged on the tree until just before the maturing burrs open; then the paper bags are replaced with cloth bags. If chestnut weevils are present in the area, the cloth bags are placed on the trees two weeks before the burrs open. The bags with their contents are collected when the mother tree has dropped most of its crop.

Chestnut pollen may be stored successfully for 10 days by collecting bagged catkins that are in full bloom, placing them in a tin can with a friction-top lid, and storing in the refrigerator. Holes should be punched in the lid to allow some ventilation. To store pollen for longer periods, place the catkins in a ventilated room to dry the nectar on them. Then shake them over a glass plate, and scrape up the pollen with a razor blade. Place the pollen in a small vial, stopper it with cotton, and place the vial in a larger vial or bottle, the bottom of which is covered with fresh calcium chloride. Because of the difficulty in germinating chestnut pollen, no tests have been made to determine its longevity when stored in this manner.

Involucres on some trees are affected by removal of the staminate catkins and by bagging, and they drop off before the stigmas are receptive. The dropping of involucres may be partially controlled by leaving the pistillate catkins intact in the bags, that is, the staminate parts of these catkins are not removed. Involucres in pollen bags do not drop even though the ovules are not fertilized. Here, however, all the catkins are intact, and a stimulative effect of the tree's own pollen may cause the involucres to remain attached and to develop eventually into sterile burrs.

Flowering Habit of Chestnut

In general, the flowers of the tree chestnuts blossom in the following order: the staminate catkins bloom first; during the period when these reach full bloom and until they brown and begin to fall, the pistillate catkins begin to bloom, and the stigmas become receptive. Stout[11] described this flower behavior as duodichogamous, having two end-season periods of pollen-shedding from the two kinds of catkins, with a midperiod for the maturity of pistils.

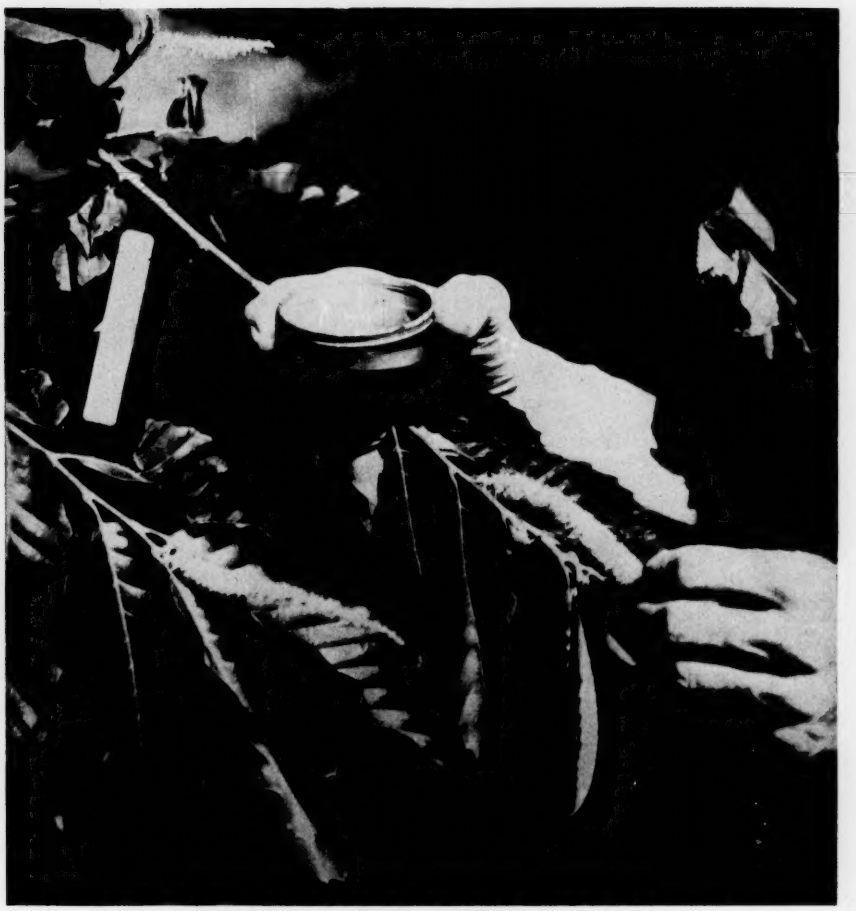
Vilkomerson[14] stated that there are two other less common types of flowering in chestnut, one in which the female flowers attain receptivity first, and another in which the male and female flowers blossom more or less together. In describing the more common flower behavior of chestnuts, she stated: "Thus we have . . . a long season of pollen shedding; and it is only during the midperiod of this season that the pistils are receptive."

Within a variety or species of chestnut, the greater abundance of pollen from the numerous staminate catkins cross-pollinates the largest number of pistillate flowers. This mass pollinization is possible because of variations in flowering of seedling trees and of different branches on the same tree, and because stigmas usually become receptive before the period of heavy pollen-shedding is over. The staminate portions of pistillate catkins are, in comparison, few and inconspicuous, and the small quantities of pollen from these catkins could not possibly pollinate large numbers of pistillate flowers.

Pollination Tests

Determining the Period of Receptivity

In 1925 the receptive condition of stigmas was ascertained from the appearance of the styles. This method was indefinite and did not provide information on the length of the receptive period. A series of tests was made to determine the relationship of the receptive pe-



HAND POLLINATION OF CHESTNUT PISTILS

Figure 2

The metal container holds mature staminate catkins. One or more of these are brushed over the styles of the female flowers on the mother tree.

riod to the beginning of anthesis, that is, the first date of flowering, of the staminate catkins.

In 1926, 62 involucres were bagged on six Chinese chestnut trees. Pollinations were made 6, 10 and 14 days after the staminates began to blossom. At 10 days there was no noticeable difference in the appearance of the styles from that noted at six days; however, the percentage of nuts set was more than four times that obtained at six days. Additional receptivity tests were made in 1928, 1931, and 1939 (Table II). In 1932, receptivity tests, with open pollination, were made on a chinkapin hybrid and on a Chinese chestnut. The bags were removed from the pistillate flowers for varying periods so that the stigmas would be open pollinated, then the bags were replaced (Table III). The results of these five tests indicate that the later the pollination is made, up to 17 days after the staminates begin to blossom, the higher the set of nuts.

Data were compiled on stigma receptivity from numerous routine hand pollinations made over a number of years on three species of *Castanea* and on two *C. mollissima* hybrids (Table IV). The various dates of pollination were translated to the number of days elaps-

ing since the beginning of anthesis for each mother tree. The data show that higher sets of nuts were generally obtained when pollinations were made twelve days after the beginning of anthesis. However, high percentages of nut-set were also obtained when pollinations were made 23 days after anthesis began on *C. crenata* and *C. pumila.*

Morning and Afternoon Pollinations

In 1930 a test was made to determine the best time of day to pollinate. Involucres on two Chinese chestnut trees were bagged, and when the stigmas were receptive, one-third of the involucres were pollinated at 9:00 a.m., 1:00 p.m., and 3:00 p.m. respectively. In 1931 a similar test was made on one Chinese chestnut tree. Combined data of the two tests gave the following results: 47 involucres pollinated at 9:00 a.m. produced 20.7 percent set of nuts, 28 pollinated at 1:00 p.m. produced 29.4 percent, and 29 pollinated at 3:00 p.m. produced 6.1 percent set of nuts. In partial explanation of these results, the greater humidity that generally prevails during the morning may induce more efficient contact and germination of the pollen grains on the stigmatic surfaces.

Self-Incompatibility of Chestnut

In 1928 a test was made to determine if pollen from either staminate or pistillate catkins would fertilize the pistillate flowers on the same tree. Twenty involucres were bagged on a Japanese chestnut and pollinated with its own pollen from staminate catkins. Seventeen additional involucres were bagged and pollinated with pollen from the pistillate catkins. No nuts set in either instance.

In 1930 additional tests were made on one Japanese and five Chinese chestnut trees. A total of 35 bagged involucres self-pollinated with pollen from staminate catkins yielded five nuts; 30 other bagged involucres self-pollinated with pollen from pistillate catkins yielded three nuts. These small sets of nuts may have been due to stray pollen entering the bags, or to self-fertilization, which rarely occurs in *Castanea.*

To test whether or not pollen tubes might reach the ovules and fertilize them if the tubes traveled a shorter distance, the styles of nine involucres on a *mollissima* × *crenata* hybrid that began to blossom on June 1, 1936, were clipped off on June 9 and pollen from the same tree was applied at once. No nuts set by this treatment. Fifteen involucres, the styles of which were not clipped, were self-pollinated but did not set nuts. Seven involucres, the styles of which were clipped, were pollinated with foreign pollen and yielded nine percent set of nuts. Six involucres, with unclipped styles, yielded 50 percent set of nuts when cross-pollinated. In another test, the styles of 11 involucres on the chinkapin hybrid S8 were clipped and self-pollinated, but no nuts set.

In 1947 another style-clipping test was made on the Chinese chestnut hybrid 016, which usually sets a small percentage of nuts from self-pollination. The styles of 15 involucres were clipped when they were bagged with neighboring staminate catkins, but no nuts set. The styles of 22 involucres were not clipped, and these produced four percent set of nuts by self-pollination. Fifteen involucres were clipped when bagged, and pollinated later with foreign pollen; these produced eight percent set of nuts. Thirteen bagged involucres were clipped just before being pollinated with foreign pollen, and these also produced eight percent set of nuts.

The foregoing results indicate that clipping the styles of involucres will not induce self-

TABLE II. Stigma receptivity tests with hand pollination

Time of Pollination (No. of days after beginning of anthesis)	No. of involucres pollinated	Nuts produced		Appearance of staminate catkins
		Number	Percent*	
	Test on 6 Chinese chestnut trees 1926			
6	24	3	4	In full bloom
10	13	7	18	In full bloom
14	14	9	21	Staminates brown-colored
	Test on 4 Chinese chestnut trees 1928			
3	13	0	0	Not fully blooming
6	14	4	10	In full bloom
9	13	5	13	Beginning to brown
14	15	14	31	Beginning to drop
	Test on 1 Chinese chestnut tree 1931			
0	13	0	0	Few blooming
3	15	2	4	Nearly full bloom
6	15	8	18	Beginning to brown
10	13	11	28	Beginning to drop
	Test on Van Fleet Hybrid 016 1939			
8	53	79	50	In full bloom; pistillates beginning to bloom
17	111	183	55	Staminates browning; pistillates in full bloom

* Based on three nuts to the burr for each involucre pollinated. The chestnut involucre normally contains three flowers, each of which, when fertilized, produces a nut.

TABLE III. Stigma receptivity tests with open pollination

No. of Involucres bagged	Bags removed (no. of days after beginning of anthesis)	Bags replaced (No. of days after beginning of anthesis)	No. of nut sets	Percentage of sets*
	Test on chinkapin hybrid			
9	2	7	0	0
18	10	12	4	22
12	15	17	6	50
	Test on Chinese chestnut tree			
11	0	3	1	3
7	5	8	8	38
16	13	Not replaced	26	54

* Based on one nut to the burr for the chinkapin hybrid, and three nuts to the burr for the Chinese chestnut, for each involucre pollinated.

fertilization of ovules, but such treatment does not prevent cross-fertilization.

Double Pollinations

In 1932 approximately 3100 involucres, cross-pollinated once, gave a set of 38 percent of fertile burrs; nearly 1200 others, pollinated twice on different days, gave a set of 46 percent of fertile burrs. The difference does not warrant climbing ladders to open the bags a second time. The efficiency of single pollinations can be increased by using pollen in the best condition, pollinating during the morning hours, and not earlier than 12 days after the beginning of anthesis of the mother trees.

Wind versus Insect Pollination in *Castanea*

A search of some of the literature dealing with the agents of pollination in *Castanea* revealed that some writers interpreted the floral morphology to indicate pollination by wind, and others interpreted it as indicating pollination by insects. Those favoring the wind-pollination hypothesis mentioned one or more of the following characteristics of chestnut flowers to substantiate their belief: Male and female flowers are borne separately; they are freely exposed to air currents; the staminate flowers produce large quantities of pollen that is light and smooth-skinned; the female flowers are inconspicuous, with no odor or nectar and no noticeable stigmatic secretion; the styles are well adapted to intercept windborne pollen—much of their surface is tacky, and supports hairs; and most wind-pollinated plants have single-seeded fruits.

Those favoring insect pollination mentioned the following to support their contention. Chestnut catkins possess odor and nectar, are colorful, and more or less erect; the pollen is not powdery but usually sticky and the grains tend to stick together on the anthers; the female flowers, although inconspicuous, are in the immediate vicinity of the conspicuous, fragrant and nectariferous male flowers; the styles are strong and stiff, with a smooth shining surface; bees and flies are the principal insects that pollinate chestnut flowers.

A third group of observers believe that both wind and insects play a part in the natural pollination of chestnuts. The literature examined did not reveal any tests made to throw light on the agents of pollination. Schad, *et al*[10] stated that the chestnut is fertilized by insects, and gave the customary reasons for their statement. However, they observed that pollen collected on the walls of bags enclosing staminate catkins, and that, under conditions of low humidity and of branches swaying in the wind, pollen would also be dislodged from unbagged staminates and carried away by air currents. It is immaterial whether catkins are erect or drooping; catkins in species and hybrids of *Castanea* vary from fully drooping to fully erect. The surface of the styles is smooth and also tacky and hairy so that pollen grains, whether insect- or wind-borne, adhere to much of the style surface. Apparently no investigations have been made to determine how much of the style surface is stigmatic, but the stigmatic surface is probably more than that at the extreme tip of the style.

Pollen grains on newly opened anthers do tend to clump, but the clumps break up as they are exposed to the air. Chestnut breeders, including those who favor insect pollination, are careful not to jar bags that contain blossoming catkins, so that the pollen will not be shaken off. When a bag is removed from

TABLE IV. *Castanea* receptivity tests; composite data from routine pollinations

Time of pollination (No. of days after beginning of anthesis)	*Castanea mollissima* 13 trees		*Castanea crenata* 3 trees		*Castanea pumila* 6 trees		*Castanea mollissima* hybrids 2 trees	
	Involucres pollinated	Nuts set*	Involucres pollinated	Nuts set*	Involucres pollinated	Nuts set†	Involucres pollinated	Nuts set*
	No.	*Percent*	*No.*	*Percent*	*No.*	*Percent*	*No.*	*Percent*
2	....	....	....	....	60	25	....	....
3	....	....	39	3	....	....	66	12
4	....	....	....	....	81	60	38	31
5	67	35	....	....	70	46	....	....
6	96	24	12	13	....	....	....	....
7	35	44	....	....	....	....	....	....
9	40	50	19	18	....	....	....	....
10	....	....	....	....	62	68	....	....
11	29	47	....	....	....	....	....	....
12	15	27	....	....	....	....	13	56
13	....	....	....	....	....	....	105	60
14	54	67	....	....	....	....	....	....
15	....	....	41	32	....	....	48	53
16	....	....	75	45	....	....	....	....
17	8	71	....	....	....	....	....	....
18	30	57	....	....	30	83	75	27
19	14	71	42	33	....	....	....	....
21	54	56	....	....	....	....	....	....
23	....	—	60	61	49	73	....	....

* Percentages of nuts set are based on three nuts to the burr for each involucre pollinated.
† Percentages of nuts set are based on one nut to the burr for each involucre pollinated.

a number of blossoming catkins and the branch is slightly jarred, a cloud of pollen grains may be seen drifting away. Blooming chestnut catkins, when shaken over a glass plate, will drop individual grains of pollen.

The writer has observed only three species of insects (*Chauliognathus marginatus, Macrodactylus subspinosus* and *M. angustatus*) that eat chestnut pollen in the orchards at Glenn Dale, Md. None of these has occasion to fly from tree to tree in search of pollen, since each blooming tree has a copious supply. In these orchards insects such as bees and flies feed on the nectar of catkins; these are comparatively rare. Thrips and leafhoppers and other small insects may be more abundant, but because of their small size, and the abundance of catkins on individual trees, they do not need to make frequent visits from tree to tree.

TABLE V. Open pollination of *Castanea* pistillate flowers protected from insects

Species or hybrid	No. of involucres bagged	Fertile burrs set		Nuts obtained	
		Number	Percent	Number	Percent*
		Involucres enclosed in netting			
C. mollissima	7	3	43	7	33
C. crenata	3	3	100	9	100
xchinkapin × *C. seguinii*	9	3	33	4	15
C. dentata × *C. seguinii*	25	8	32	14	19
Totals and averages	44	17	39	34	33
		Involucres enclosed in cheesecloth			
C. mollissima	10	5	50	11	37
C. crenata	2	2	100	6	100
xchinkapin × *C. seguinii*	4	1	25	1	8
C. dentata × *C. seguinii*	21	7	33	8	13
Totals and averages	37	15	41	26	23
		Check: Involucres open pollinated			
C. mollissima		10		27	90

* Based on three nuts per burr for each involucre.

TABLE VI. Open pollination of Chinese chestnut flowers; staminate flowers removed

Tree number	Involucres bagged	Fertile burrs set		Nuts obtained	
	Number	Number	Percent	Number	Percent*
	Involucres bagged until stigmas were receptive, then bags removed				
R 59	4	4	100	8	67
R 66	7	4	57	9	43
A 58	5	5	100	7	47
P 59	2	2	100	6	100
Totals and averages	18	15	83	30	56
	Checks: male and female flowers intact and not bagged				
R 59		9		25	92
R 66		10		27	90
A 58		8		21	87
P 59		8		23	96
Totals and averages		35		96	91

* Based on three nuts to the burr for each involucre.

The rarity of insects that make frequent visits from tree to tree in the Glenn Dale, Md., orchards, and the fact that insignificant quantities of dry pollen would adhere to their bodies, eliminate them as an important agent in setting the large crops of nuts obtained there. Large insects such as bees, flies, and weevils do, however, play a secondary role in the cross pollination of chestnuts. Crawling over blooming catkins, they dislodge pollen from the anthers so that it is carried away as a dust by wind and convection currents.

The writer designed various tests to determine if the pollen of *Castanea* is windborne, and also to determine the extent that such windborne pollen fertilizes female flowers. In 1946, greased slides were set out over night under and between blooming chestnut trees at Glenn Dale, Md. When these slides were examined under a microscope, individual grains of chestnut pollen were found on all the slides.

In 1947, pistillate flowers not yet receptive on several chestnut trees were enclosed in cheesecloth and mosquito netting. All male parts of the catkins were emasculated at this time. The sets of fertile burrs and nuts obtained (Table V), therefore, must have resulted from airborne pollen passing through the meshes of the cloth and netting. The check in this test was a random selection of ten burrs from open pollination of a Chinese chestnut tree; the number of nuts in these burrs was recorded.

In another test, made in 1947, all staminate parts of catkins were emasculated, and the involucres were enclosed in kraft paper bags. When the stigmas were at maximum receptivity, the bags were removed and not replaced. Here, as in the preceding test, insects would not be attracted to the female flowers, and if pollination of these occurred, it must have been by means of wind. The fact that the involucres were bagged during the early part of the receptive period probably accounts for the lower percentages of nuts set in some instances (Table VI). The checks in this test were unaltered flowers subject to open pollination on the test trees.

In these tests, emasculating chestnut flowers so that insects would not be attracted to them and further protecting the pistillate flowers from insects by cheesecloth or netting, did not in any case result in a complete crop of sterile burrs. Since cheesecloth and netting partly prevent pollen grains from passing through to the pistils, these barriers would certainly stop pollinating insects.

The writer concludes from the results of the foregoing tests, that (a) chestnuts are largely wind pollinated; (b) wind pollination is the natural and normal method; (c) wind pollination is more efficient than insect pollination in species of *Castanea* where the flowers are separated by sex; (d) insects are not essential for pollination of chestnut.

(To be continued)

THE GENETICS OF A NEW HAIR DEFICIENCY, FURLESS

In the House Mouse

EARL L. GREEN*

NEW genes which affect the color, pattern, form, amount, and distribution of hair in the house mouse are of interest, first, because of their prospective usefulness in expanding the known linkage relationships and second, because of their possible contribution to the knowledge of the normal development of hair. The purpose of this paper is to record the occurrence of a new hair deficiency, called "furless," and to record the evidence that furless depends upon an autosomal recessive gene (symbol *fs*) which is neither an allele of, nor closely linked with hairless, *hr*.

Two female mice (litter mates) with notably deficient pelages were found in some unpedigreed stocks maintained in the Department of Zoology in the summer of 1951. No mice of this sort had previously been seen, nor had there been any recent introductions of mice from elsewhere. Shortly after they were first observed, each developed a normal appearing coat of fur. Still later this coat was shed. The coats thereafter remained distinctly deficient for a period of more than a year.

Description of Furless

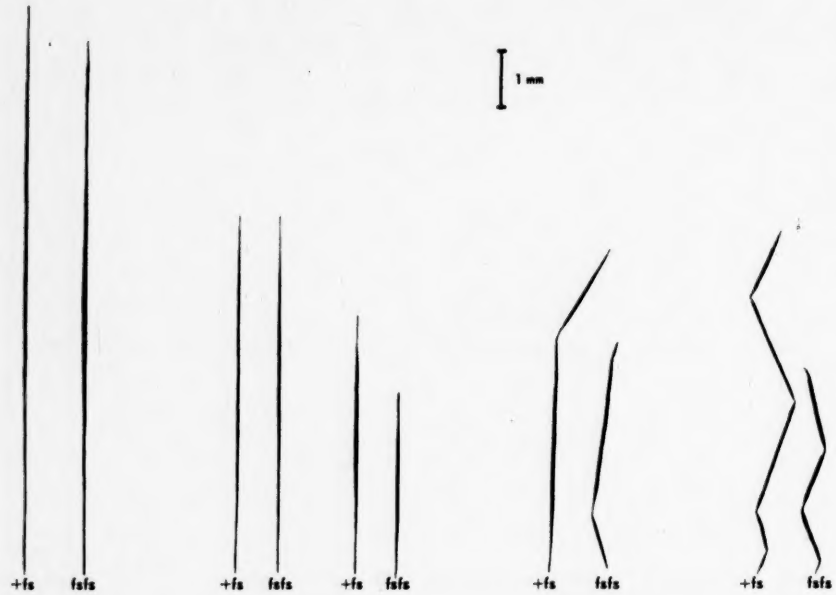
The following description of the external appearance is based on furless mice with wildtype coat color subsequently produced during breeding tests. It applies, with minor modifications, to non-agouti, cinnamon, brown, and pink-eye mice. Two days after birth, the furless mice may be distinguished from normal by very short or missing vibrissae. By six days, the coat as a whole is different in appearance, in not having the yellowish cast of the normal agouti mice. The difference in coat color disappears about the sixteenth day. By the tenth day, the furless mice are smaller in size than their normal litter mates. This difference may persist throughout life, though it is less marked by three months. About 19 days of age, the furless mice begin to lose their first coat of fur. This is noticeable as a thinning of the hair on the head between the eyes. Gradually the depilation proceeds over the entire body. About the sixth week, a new coat is produced but it persists for only a short time. The mature mice show varying degrees of persistence of fur, almost never being as completely devoid of fur as are "hairless" mice.

The four kinds of hairs, guard hairs, awls, auchenes, and zig-zags, normally present in the mouse coat are also present in the "furless" coat when the coat reappears after the first shedding. The percentages of the four kinds are also about the same, although there may be more auchenes (16 percent) and fewer awls (10 percent) than in the normal coat. The "furless" coat is quite markedly different from normal in that the hairs of all types are shorter (Figure 3).

Genetics of Furless

The original "furless" females were mated with a male of the C57BL/10 strain. In six F_1 litters 41 normal males and females were produced (Table I, mating 1). In 55 F_2 litters, 354 normal and 97 furless mice were produced (mating 2). These numbers do not differ significantly from the expected three

*Department of Zoology, The Ohio State University, Columbus 10. It is a pleasure to record my indebtedness to Mr. Ronald E. Fowler who kindly made the two original furless mice available to me, to Dr. Herman B. Chase who supplied me with hairless mice from his stock, and to Mr. Edwin P. Les for the preparation of the figure.



NOMAL AND FURLESS HAIR TYPES

Figure 3

The four kinds of hair normally present in the mouse, are also present in "furless," but "furless hair" (at right) are markedly shorter than in the normal coat.

normal to one furless if furless is due to an autosomal recessive gene.

To test the hypothesis that furless is caused by a recessive gene, F_2 normal mice were mated with furless mice. Altogether 82 such mice, including 17 males and 65 females, were tested. If the hypothesis is correct, one-third of the F_2 mice should be homozygous, and two-thirds should be heterozygous carriers of furless. There were 24 which tested as homozygotes and 58 as heterozygotes, in satisfactory agreement with expectation (Table II, matings 3 and 4).

The test of a normal mouse for homozygosity by mating with furless implies a risk of misclassification which is small or large depending upon the criteria used. If a test mating $A— \times aa$ yields one or more offspring of the *aa* sort, it follows that the tested mouse is *Aa*. On the other hand if the test mating yields n normal mice, the tested mouse may be *AA* or it may be *Aa*, although the probability for it to be *Aa* decreases as n increases. More exactly the probability for n normal offspring given a mating of the sort $Aa \times aa$ is $(\frac{1}{2})^n$. This probability may be made as small as desired by increasing n. It was decided that a test mating $A— \times aa$ would have to produce at least seven normal offspring before the tested mouse would be classified as *AA*. This reduced the probability of misclassification to 1/128 or less. Of the 24 mice classified as *AA*, 7 were so classified by having produced

TABLE I. Results of mating furless and normal mice

	Mating Sire	Dam	Number of matings	Number of litters	Generation	Normal ♂♂	Normal ♀♀	Furless ♂♂	Furless ♀♀	Total
1.	Normal	Furless	2	6	F_1	16	25	0	0	41
2.	F_1	F_1	16	55	F_2	170	184	55	42	451
					Obs.	354		97		
					Exp. 3 : 1	338¼		112¾	$\chi^2 = 2.93$	

7 normal offspring, 4 by 8 normal offspring, 4 by 9 normal offspring, and the remaining 9 by having produced 10 to 15 normal offspring.

In subsequent generations, furless appeared in proportions which were consistent with the interpretation that the furless type depends upon an autosomal recessive gene (Table III, matings 5 to 7). The furless character apparently depends upon a recessive autosomal gene, which may be called furless, symbol *fs*.

Test for Allelism of Furless and Hairless

Since furless *fs* and hairless *hr* have similar phenotypic effects, it is pertinent to see if they behave as alleles. In nine litters produced by matings of ♂ *hrhr* with ♀ *fsfs*, 44 normal mice were produced (Table IV, mating 8). These normal mice are presumptive evidence against *fs* and *hr* being alleles, since the normal mice so produced would have to be *fs*/*hr*, on the hypothesis of allelism. In view of the fact that cases are known of two alleles restoring the wild type in heterozygous condition, this cannot be regarded as conclusive evidence against allelism.

TABLE II. Results of testing F_2 normal mice for furless

Mating Sire	Dam	Tested and found to be: Homozygous	Heterozygous	Total
3. F_2 normal	× Furless	5	12	17
4. Furless	× F_2 normal	19	46	65
Obs.		24	58	82
Exp. 1:2		27 1/3	53 2/3	$\chi^2 = 0.610$

If the F_1 normal mice are *fs*/*hr*, the F_2 generation should consist of 2/4 *fs*/*hr* (normal), ¼ *fs*/*fs* (furless), and ¼ *hr*/*hr* (hairless). On the other hand if *fs* and *hr* are not alleles and not linked, the F_1 mice are + *fs* + *hr*, and the F_2 generation should consist of 9/16 +— +— (normal) 3/16 *fs fs* +— (furless), 3/16 +— *hr hr* (hairless), and 1/16 *fs fs hr hr* (hairless?). In the F_2 generation there were 184 normal, 67 furless, and 68 hairless mice (Table IV, mating 9). The question is whether this result represents an 8:4:4 ratio (if *fs* and *hr* are alleles) or a 9:3:4 ratio (if *fs* and *hr* are independent non-alleles). Tested against the 8:4:4 ratio the observed numbers yield a χ^2 which is larger than the critical value of χ^2 at the five percent level of significance for two degrees of freedom, but when tested against the 9:3:4 ratio there is satisfactory agreement between the observed and expected numbers. This is evidence in favor of 9:3:4 being the true ratio. However, an important question is whether the sample size is large enough to reduce, to a selected low level, the probability of accepting the 9:3:4 ratio as true if in fact the 8:4:4 is correct and also large enough to reduce to a low level the probability of accepting the 8:4:4 ratio as true if the 9:3:4 ratio is correct. There must exist a set of numbers a_1, a_2, a_3, called the "ambiguous segregation numbers," such that the probability of their arising is the same under both the 9:3:4 and the 8:4:4 ratios as expectations. The sample size n $(= a_1 + a_2 + a_3)$ should be large enough to reduce the probability of getting a_1, a_2, and a_3 to, say, five percent under either hypothetical ratio. It may easily be determined that n should exceed 448 mice to satisfy this requirement. In view of the fact that the F_2 generation contained fewer than 448 mice, the 9:3:4

TABLE III. Results of matings in generations 3 to 6. *fs* = furless

Mating Sire	Dam	Number of matings	Number of litters		Normal + —	Furless *fs fs*	χ^2
5. *fsfs*	× *fsfs*	13	30		0	134	
6. *fsfs*	× +*fs*	18	51	Obs.	158	127	
+*fs*	× *fsfs*			Exp. 1:1	142½	142½	3.37
7. +*fs*	× +*fs*	2	5	Obs.	21	5	
				Exp. 3:1	19½	6½	0.46

TABLE IV. Results of mating furless with hairless mice

Mating Sire	Dam	Number of matings	Number of litters	Generation	Normal ♂♂	Normal ♀♀	Furless ♂♂	Furless ♀♀	Hairless ♂♂	Hairless ♀♀	Total
8. Hairless	× Furless	5	9	F_1	23	21	0	0	0	0	44
9. F_1	× F_1	13	51	F_2	95	89	35	32	42	22	319
			Obs.		184		67		68		$\chi^2 =$
			Exp. 9:3:4		179 7/16		59 13/16		79 3/4		2.711
			Exp. 8:4:4		159 1/2		79 3/4		79 3/4		7.533
			Exp. 9:3:3		191 2/5		63 4/5		63 4/5		0.723

ratio for two pairs of genes should not be regarded as established, though the data favor it in preference to the 8:4:4 ratio for allelic genes.

It should be noted that the data are fitted better by a 9:3:3 (or 3:1:1) ratio which may exist if the double homozygotes *fs fs hr hr* do not survive.

The crucial test of the hypothesis of *fs* and *hr* being alleles consisted of making two kinds of test matings. The first consisted of matings of F_2 *hr hr* males from mating 9 with known *fs fs* females. If *fs* and *hr* are alleles, such matings should produce mice with the normal phenotype exclusively as in mating 8. If *fs* and *hr* are not alleles, some of the *hr hr* males should also carry *fs*, either as *fs fs* or as + *fs*. The second test mating consisted of matings of F_2 *fs fs* females from mating 9 with known *hr hr* males. If *fs* and *hr* are alleles, these matings should produce only normal offspring, whereas if *fs* and *hr* are not alleles, some of the *fs fs* females should turn out to be carriers of *hr*.

Of seven F_2 *hr hr* males so tested, four were shown to transmit *fs* and the normal allele of *fs* and so were shown to be + *fs hr hr* (Table V, mating 10). Of nine F_2 *fs fs* females tested, five were shown to transmit *hr* and the normal allele of *hr*, and so were shown to be *fs fs* + *hr* (mating 11). There is no doubt, therefore, that *fs* and *hr* are not alleles.

It may be further noted that no *hr hr* males turned out to be *fs fs hr hr*. Several hairless males which had defective vibrissae and somewhat wrinkled skin, as contrasted with nearly normal vibrissae and smooth skin of other hairless males, were set out for test matings. These were presumed to be *fs fs hr hr*, but all died within a few days after weaning. Pending a more thorough investigation of this point, it must be stated that the combination *fs fs hr hr* has not yet been identified with certainty.

Test for Linkage of Furless and Hairless

The data on the assortment of *fs* and *hr* in the F_2 generation (Table IV, mating 9) indicate that *fs* and *hr* are at least not closely linked. In fact, the numbers of normal and furless mice in the F_2 generation deviate from the ¾ and ¼ expected if there is no linkage in the direction opposite to that expected if there is linkage.

To test for the existence of linkage further than provided by the F_2 data, a few single backcross matings of two types were made. One type consisted of mating repulsion heterozygous ♂♂ + *hr*/*fs* + with ♀♀ known to be *fs* +/*fs hr*. The other consisted of mating repulsion heterozygous ♀♀ with ♂♂ known to be + *hr*/*fs hr*. The results are shown in Table VI, matings 12 and 13. There is no significant departure from expectations based upon random assortment in either mating. The maximum liklihood estimates of the crossover frequencies are 0.68 ± 0.30 and 0.56 ± 0.17, neither of which departs significantly from one-half. In fact, both deviate from one-half in the wrong direction to indicate linkage of *fs* and *hr*.

Summary

A new hair deficiency, called "furless," in the mouse depends upon a single recessive autosomal gene, symbol *fs*. The new gene is not an allele of hairless, *hr*, nor linked with it.

TABLE V. Results of testing F_1 hairless males and F_2 furless females (*fs* = furless, *hr* = hairless)

	Mating Sire	Mating Dam	Number of Matings	Test progeny contained: Neither	*fsfs*	*hrhr*	Both
10.	F_2 *hrhr*	× *fsfs*	7	3	4	0	0
11.	*hrhr*	× F_2 *fsfs*	9	4	0	5	0

TABLE VI. Test for linkage of *fs* and *hr*

	Mating Sire	Mating Dam		Normal	Furless	Hairless	χ^2
12.	$\frac{+\ hr}{fs\ +}$	× $\frac{fs\ +}{fs\ hr}$	Obs.	14	11	7	0.54
			Exp. 3:3:2	12	12	8	
13.	$\frac{+\ hr}{fs\ hr}$	× $\frac{+\ hr}{fs\ +}$	Obs.	39	11	51	0.25
			Exp. 3:1:4	37⅞	12⅝	50½	

VARIABILITY OF THE BANDING PATTERNS OF GIANT CHROMOSOMES

ATIF SENGÜN

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IT has been shown[3, 4, 7] that the banding patterns of the same giant chromosomes of *Chironomus*-larvae are not the same in the cells of different tissues and that they differ from cell to cell in the same tissue. Preliminary experiments (unpublished) show that there is greater variability in the pattern of giant chromosomes of Diptera having a longer larval life span than those which have a shorter larval period. The hypothesis was advanced that diversity of disc patterns is due to differences in the function of the cells having giant chromosomes, to the ontogenetic stage of the given chromosomes in different tissues and also to external factors. These results were criticized by Beermann,[1] Slizynski[9] and by Pavan and Breuer.[6] Although Beermann accepts the occurrence of differences in the disc patterns of giant chromosomes as due to different functional and external factors, he considers these differences to be of secondary importance and not essential characters of the chromosomes; while according to Slizynski[9] and to Pavan and Breuer[6] the same giant chromosome shows identical structure in all cells with polytene chromosomes.

In examining giant chromosomes one always observes that the same chromosome has different banding patterns in most of the cells of any one tissue. This may be due partly to the technique used. Primarily however, it must be attributed to the state of the chromosomes themselves, since the difference between the homologous chromosomes of different tissues or the homologous chromosomes of the same tissue of old and young larvae are greater than those observed in the same tissue of the same individual.

STRETCHED CHROMOSOMES

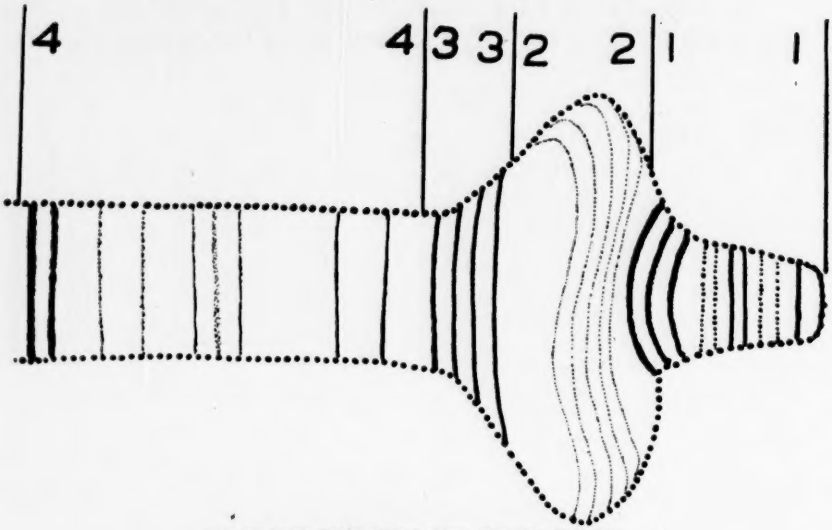
Figure 4

The number of cross bands on the same chromosome stretched to various degrees is shown. There is apparently no positive relation between the length to which a chromosome is stretched and the number of cross bands of a given region.

All investigators, as stated by Painter,[5] have observed those structural differences without attributing any importance to them. Table II in the publication of Beermann[2] shows that some bands are present in a region of a chromosome which are lacking in the same region of a homologous chromosome of another cell of a different tissue. Most investigators have ignored this variability and have attempted to find chromosomes with completely similar patterns. In fact some chromosomes have been found with identical structures. But we must not forget that there are always other chromosomes with different banding patterns. It is also possible to choose chromosomes with different disc arrangement. This has been shown in Table I. Since we do not always find the same number of cross bands and since so far there is no proof that the cross band number is constant, we must assume that we are dealing with a variable structure.

TABLE I. Variation of cross band numbers in a certain chromosome region

Number of cross bands	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Number of salivary gland chromosomes examined		2	4	14	8	26	38	34	28	14	16	14	18	2	2	2	4	1	1	0	1
Number of chromosomes examined from tracheal cells	1	5	3	2	3	0	2	1													



SALIVARY CHROMOSOME CROSS BANDS

Figure 5

A reconstructed chromosome region from the salivary glands of *Drosophila repleta* is shown. This "ideal" reconstructed chromosome region has 31 cross bands, but this number was not found in any of the 229 actually examined.

Therefore we must first find the average value for the number of cross bands of a given chromosome. If for the moment we take the validity of the polytene theory for granted and obtain an average value for cross bands, using the method generally applied in the construction of chromosome maps, it is to be expected that the average values obtained must be identical or nearly identical for a given chromosome in all the tissues of a larva.

Method

In the present experiment *Drosophila repleta* was used instead of *Chironomus*. A chromosome region was chosen which is not difficult to recognize and the number of cross bands were drawn or counted in 229 chromosomes from large or small nuclei of the salivary glands of different larvae in the following way: The chromosome region was divided into four arbitrary sections and the cross bands in each section were counted. The region among 229 chromosomes examined, which had the highest number of cross bands was selected for the construction of an ideal region map as shown in Figure 5. By a statistical method it was attempted to determine how many chromosome regions of these 229 samples corresponded in the number of cross bands to the scheme in Figure 5, i.e. the ideal map constructed. As shown in Table I only one chromosome has 26 crossbands in this region whereas in the "ideal" reconstructed chromosome shown in Figure 5 this region has 31 cross bands. The ideal number of cross bands was never found in any of the 229 chromosomes examined. But many of these regions have cross bands in numbers varying from 9 to 18. The average value of cross bands in this zone is $M \pm 3m = 14.98 \pm 0.60$. Under these circumstances are we justified in affirming that the reconstructed ideal chromosome map seen in Figure 5 represents the real situation? We have no reason to accept the fact that a cross band which is visible in some of the chromosomes must also exist in all others.

Many investigators are of the opinion that when there is a variability, it is due to the degree of stretching of the chromosome. A comparison of the number of cross bands in a chromosome region which are stretched to the same degree, as judged by length, or which show differences in the degree of distention demonstrates that there is no positive relation between the length and the number of cross bands of a given chromosome region (Figure 4). But the chromosomes of smaller nuclei have fewer cross bands than those of the larger nuclei of the same gland. It may be

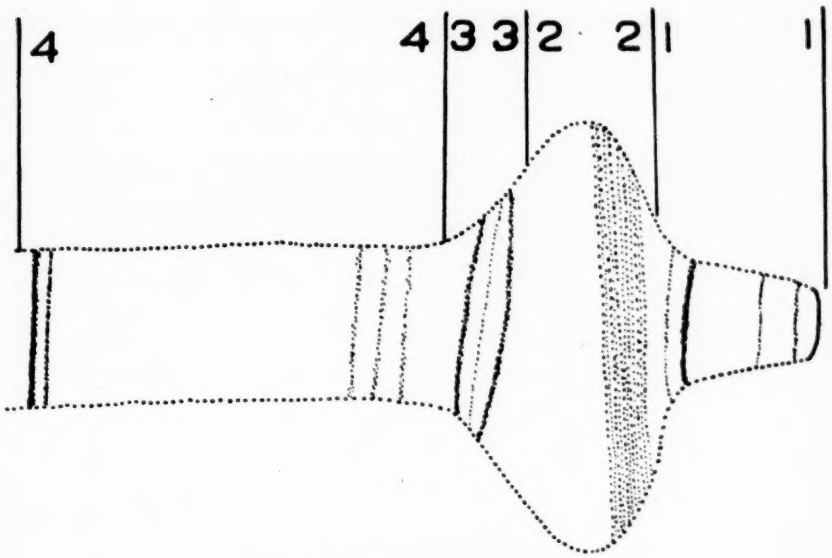


Figure 6

A reconstructed chromosome region as in Figure 5, from the tracheal cells. This region drawn from 17 chromosomes has fewer cross bands than that of the same chromosome region of an "ideal" salivary gland chromosome.

added that only chromosomes lying flat on one plane, where none of the bands could miss being observed, were taken into consideration in making the counts.

Some large tracheal cells have giant chromosomes closely resembling those of the salivary gland. Using the same method as described above, an "ideal" map of the same region of a homologous tracheal cell chromosome is reconstructed (Figure 6). This reconstructed chromosome region drawn from 17 chromosomes has fewer cross bands than that of the same chromosome region of an "ideal" salivary gland chromosome (Table I). The average value for the cross bands of this chromosome region is $M \pm 3m = 8.89 \pm 1.41$. If we compare this average value with that of the constructed chromosome of the salivary glands (14.98 ± 0.60), we find a great difference which is statistically confirmed. The number of cross bands is generally reduced in sections 1 and 4 and in section 3 many chromosomes have 3 cross bands instead of 4. This is typical of the salivary gland cells. In section 2 the cross bands are often hard to distinguish since they appear as one broad diffuse band regardless of the extent of chromosome distention. But the number of cross bands in the chromosomes of both the salivary glands and the tracheal cells show overlapping values. In the overlapping value region we find that many chromosomes of both tissues have identical numbers of cross bands. But we have to bear in mind that besides these chromosomes that fall in the overlapping value region, there are many chromosomes in the salivary gland cells that have a different disc pattern with a higher number of bands.

Conclusions

It is concluded that the average values for the number of cross bands are different since it has been statistically established that there is a variability in the number of cross bands of a given region of homologous chromosomes of the same tissues and also of different tissues having giant chromosomes. On the other hand there are chromosomes in different tissues which show a similar pattern in the overlapping value region. But there is no reason to accept these chromosomes as representative of the chromosomes of different tissues.

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Rapid Identification of Recessive-White Onion Bulbs by Use of Ammonia Fumes

Elmo W. Davis*

A WHITE onion bulb can be the result of two kinds of genes for white—one a dominant inhibitor and the other a recessive white. In the past it was necessary to subject a white onion bulb to a genetic test to determine which pairs of genes were present. This note suggests a way to differentiate bulbs which are white because of a homozygous recessive gene from those which are white because of a dominant inhibitor.

Rieman[2] first demonstrated the occurrence of two kinds of genes for white. Clarke, Jones and Little[1] substantiated these results but showed that the genes differentiating red and yellow pigments were not allelomorphic with the recessive white gene as Rieman[2] had presumed.

The dominant *C* gene is a basic color factor necessary for the production of any pigment. All *cc* plants produce white bulbs. The dominant *R* gene in the presence of dominant *C* is responsible for the production of a red pigment; its allele *r* is responsible for yellow in the presence of dominant *C*. The inhibitor factor *I* first reported by Rieman[2] is partially dominant over *i*. Plants having the genotype *Ii* produce some color, particularly around the neck of the bulbs, but are readily distinguished from red and yellow. The heterozygous plants *Ii* cannot always be distinguished with certainty from the homozygous *II* white.

A variety of onions homozygous for red has the genotype *iiCCRR;* for yellow, *iiCCrr;* and for recessive white, *iiccRR*, *iiccRr* or *iiccrr*. All *II* plants are white and are designated as dominant white.

Previously it was not possible to distinguish with certainty dominant whites from recessive whites without resorting to genetic tests. However, by the aid of a simple chemical test recessive whites are readily distinguished from dominant whites.

Rieman[2] reported that the yellow pigment in onion bulbs is a flavone which ranges in color from pale ivory to deep yellow. It is found in the cell sap. An increase in the number of hydroxyl groups present in flavone produces a deeper yellow color. The practically colorless flavone is produced in the dominant *II* white onion but not in the recessive white onion. If the outer fleshy scales of any onion bulb are treated with concentrated ammonia fumes a chemical change in the flavone, if present, takes place and a deeper yellow is produced. Recessive white onions show no reaction, whereas a dominant white onion will show a patch of yellow in the outer fleshy scales. The test can be hastened if the outer scale is finely cross-hatched with a sharp knife to release some of the cell sap before treatment with the ammonia vapor. Only a few seconds is necessary for the color to develop if flavone is present.

This method of detecting a recessive white onion will save at least two generations in determining the genetic constitution of a white onion.

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AN INHERITED RETINAL DEGENERATION IN THE MOUSE

KATHARINE TANSLEY*

PYKNOTIC CELLS

Figure 7

Degenerate pyknotic cells are shown in the outer nuclear layer. At 14 days of age pyknotic cells were present in 60 percent of the albino strain animals examined. This degeneration begins in the central retina and spreads rapidly throughout the outer nuclear layer. This is a higher magnification of Figure 9*B*. The following lettering applies to all figures: S.—Sclera. CH.—Choroid. P.E.—Pigment epithelium. O.L.R.—Outer limbs of rods. I.L.R.—Inner limbs of rods. R.—Rods O.N.L.—Outer nuclear layer. O.F.L.—Outer fibre layer. I.N.L.—Inner nuclear layer. I.F.L.—Inner fibre layer. G.C.—Ganglion cells. O.N.F.—Optic nerve fibres. P.C.—Pyknotic cells.

HEREDITARY degeneration of the retina has been described in rats,[1] mice[12, 6] and dogs.[10] In rats and dogs the retinal lesion has been shown to be inherited as a recessive Mendelian character. The present paper outlines the development of the lesion in a stock of mice originally obtained from Dr. R. Brückner, and describes breeding experiments designed to determine its mode of inheritance.

When fully developed this lesion closely resembles that described by Keeler[8] under the name "rodless retina" but the early stages in the two conditions appear to be quite unlike. According to Keeler's description "rodless retina" is a condition of arrested development. The retinae develop normally until six or seven days after birth except for a reduction of mitotic activity compared with the normal eye. There is no more development there-

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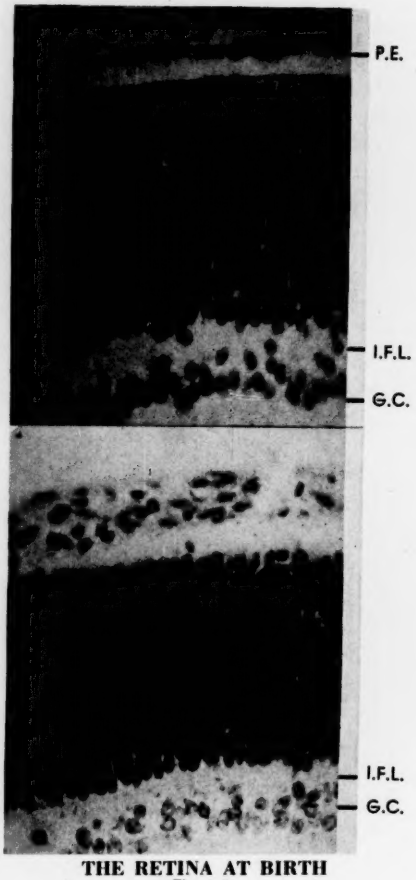
after and the rods never appear. No degenerate cells were observed. The end result was a retina lacking its outer layers—rods, outer nuclear and outer fiber layers. Since Keeler[2] published his classic description of "rodless retina" in the mouse it has been customary to describe any mouse strain with a retina from which the outer layers are missing as "rodless" and to assume that it carries Keeler's gene. This does not appear to be a safe assumption. At least one of the strains so described, the P/Jax strain shows the same normal retinal development followed by degeneration described here in the Swiss stock and, so far as the retina is concerned, is genetically the same.

The mice belong to an inbred albino stock and some 10 generations have now been reared at the Institute of Ophthalmology from three affected animals (two males and one female). No abnormality has been observed in them other than the retinal lesion. No cataracts have appeared. There is no known relationship between this stock and those investigated by Keeler, Cohrs[3] or Hopkins[5] although the possibility cannot be ruled out, particularly where Cohrs' and Hopkins' mice are concerned. (Brückner, personal communication).

Materials and Methods

The eyes of 95 animals were examined at ages varying from birth to 15 months. The animals were killed by breaking the neck and the eyes immediately removed and fixed in either Zenker's or Kolmer's fluid. The anterior part of the eye including the lens was removed from all animals over 11 days of age during dehydration and the back of the eye embedded in paraffin. Sections were cut at 6μ and stained with haematoxylin and eosin, Mallory's phosphotungstic acid haematoxylin, the azan modification of Mallory's triple connective tissue stain, Heidenhain's iron haematoxylin counterstained with aniline blue and orange G or with phloxine and Feulgen's method. All sections from the affected stock were compared with two normal strains of the same age.



THE RETINA AT BIRTH
Figure 8

A—Section through a normal mouse retina at birth. *B*—Section through the retina of a mouse from the affected stock at birth. No differences are found at this stage of development. Zenker fixative: Feulgen. (× 300).

Results

At birth there was no difference between the eyes of the affected stock and those of normal mice. In both cases the retina was immature, consisting of the optic nerve fiber layer, the ganglion cells, the inner fiber layer and a thick layer of undifferentiated cells showing many mitoses along its outer edge (Figure 8*A* and *B*). Up to 13 or 14 days the affected retinae appeared to develop normally although the first appearance of the outer fiber layer and of both inner and outer limbs of the rods (there are no cones in the mouse) may have been slightly delayed. By 13 days, how-

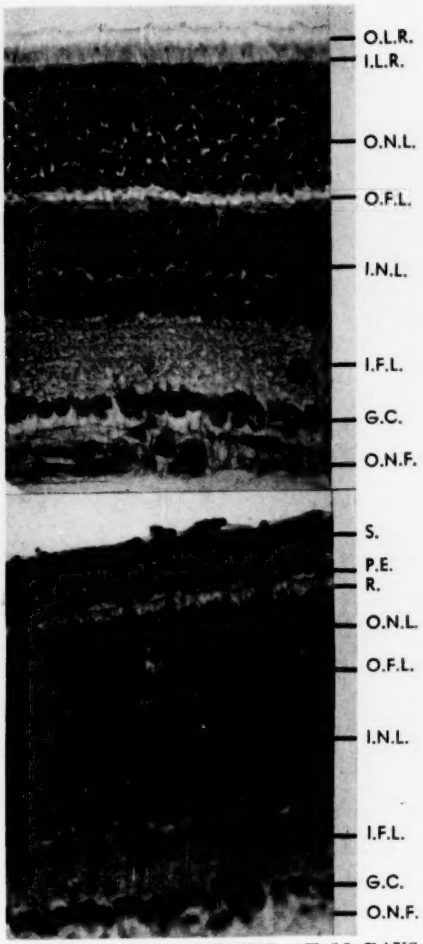
ever, the affected retinae were indistinguishable from those of normal animals of the same age except for some degenerate cells in the outer nuclear layer in a few eyes (Figures 7 and 9*A* and *B*).

At 14 days of age pyknotic cells were present in 60 percent of the affected animals examined. This degeneration began in the central retina and spread rapidly throughout the outer nuclear layer until, at 16 days, the layer was reduced to a fraction of its normal width and most of its cells were dead (Figure 10*A* and *B*). At this stage the rods had lost their characteristic structure and gradually disintegrated until, by about the twenty-first day, nothing but debris could be seen between the external limiting membrane and the pigment epithelium. At four weeks nothing remained of the rods or of their nuclei (Figure 11).

At about the same age the external limiting membrane could only be seen in places, and some parts of the pigment epithelium looked swollen and abnormal. From this stage onwards the development of the lesion became more irregular, often progressing faster in one eye than in the other or even in different parts of the same retina. Sometimes the retina remained for months at the stage shown in Figure 11. Where further changes occurred these consisted of gliosis and disorganization of the retinal layers with some adhesion between retina and choroid. These are changes which are not specific to this disease but which tend to be seen in any condition in which the outer part of the retina is lost. The choroid itself might also be affected, becoming either enormously thickened or almost non-existent. But in every eye examined there were parts where the inner layers of the retina and the choroid were normal in appearance.

The retinal lesion appears to be inherited as a Mendelian recessive character. All the adult offspring of two matings of affected females to normal males of the A/Gr strain and one mating of an affected male to a normal female (14 mice in all) had normal retinae. Three matings between F_1 heterozygotes produced 15 normal and six affected animals. Two matings of affected animals to their heterozygous offspring resulted in five normal and four with degenerate retinae. Matings between affected homozygotes have always produced 100 percent degenerate retinae. Altogether about 70 such offspring were examined after they were old enough to show the disease for certain.

A mating was made between an affected male and a female of the P/Jax strain obtained through Dr. H. Grüneberg. Adult P/Jax mice have rodless retinae and the strain has, therefore, been thought to carry Keeler's gene, an assumption based on the phenotypic similarity, not on breeding experiments. The result of this mating was six offspring all with affected retinae so it would seem that the P/Jax strain and the one considered here carry the same gene for degenerate retina. It does not, of course, follow that these two strains carry Keeler's gene.



NORMAL AND AFFECTED AT 13 DAYS
Figure 9

A—Section through the retina of a normal mouse at 13 days. Zenker. Azan. *B*—Section through the retina of a mouse from the affected stock at 13 days. Pyknotic cells begin to appear in affected individuals. Kolmer fixative: Heidenhain's iron haematoxylin and phloxine. (×480).

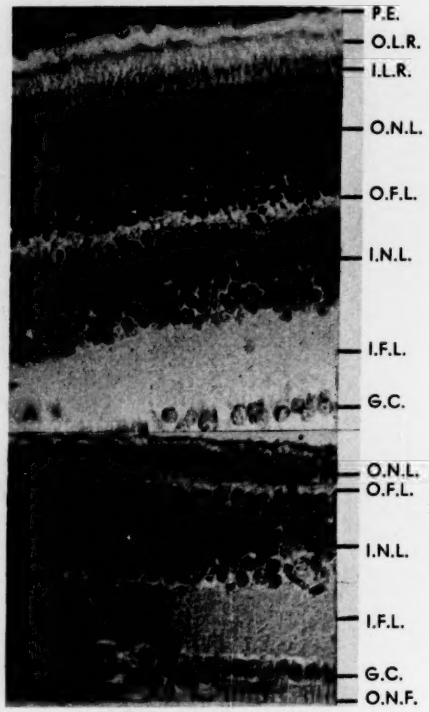
Some observations were also made on the development of the lesion in the P/Jax mice. This strain was very hard to breed and only a few young mice were available for study. The retina developed normally up to 11 days. At 14 days there was heavy degeneration in the outer nuclear layer and the rods showed some disintegration. Unfortunately no mice

between 11 and 14 days could be examined before the stock died out. Five grown mice between the ages of four and 10 months showed the typical adult condition (Figure 11).

Discussion

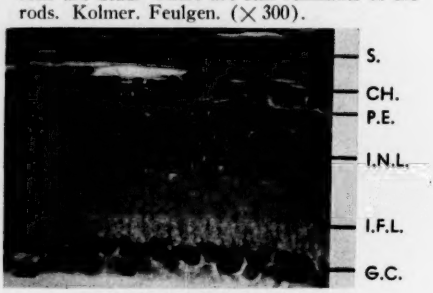
It appears that the changes which result in the disappearance of the visual cells in this condition must be a fairly direct manifestation of the mutant gene. They are constant and regular and are always the same in each eye. The later changes, those affecting the inner retina, pigment epithelium and finally the choroid show much more variation in their extent and time of appearance. It was thought, therefore (Tansley), that these were probably secondary changes only indirectly related to the fundamental genetic abnormality. This seemed particularly likely since similar changes, particularly of the inner retina, follow disappearance of the visual cells no matter from what cause. However, an extremely interesting observation recently published by Noell[9] suggests that the pigment epithelium changes at least, may also be fairly directly associated with the action of the mutant gene. Noell found that small intravenous doses of monoiodoacetate given to rabbits, cats and monkeys produced degeneration of the rods followed by degeneration of the cones in cats and monkeys if the dose were repeated. A further increase in dose caused degeneration of the pigment epithelium. In Noell's experiments the degenerative changes were sometimes so slow that it was possible to make out which part of the rods were the first to be affected. In these cases the outer limbs went first, then the inner limbs and finally the nucleus. In the diseased mice the degenerative changes occur very rapidly at a stage when the rods are not fully mature and it is impossible to be certain what part of the cell is the first to be affected. In such young retinae the nuclear pyknosis is much more striking and easily recognizable than outer limb changes. This is the first positive sign of the onset of degeneration. Of course this does not necessarily mean that the nucleus is really affected before the outer limb. Iodacetate is a well-known enzyme poison which prevents normal glycolysis and Noell's observations suggest that the hereditary disease is due to a lack or abnormal functioning of one of the enzymes concerned with retinal glycolysis.

The disease described here appears to be very similar to the mutation which is responsible for hereditary retinal degeneration in rats and dogs and possibly retinitis pigmentosa in man. In mice, rats and dogs the condition is inherited as a Mendelian recessive character and so is one type of retinitis pigmentosa. In animals the first effect is degeneration of the rods at the time when they begin to function. In man and dogs there is always a long history of night blindness—a symptom of rod deficiency. In addition, both in man and the dog the



RETINAE AT 16 DAYS
Figure 10

A—Section through the retina of a normal mouse at 16 days. This retina is fully differentiated. Zenker. Azan. *B*—Section through the retina of a mouse from the affected stock at 16 days. The outer nuclear layer is much reduced in width and most of its remaining cells are dead. There are still remnants of the rods. Kolmer. Feulgen. (× 300).



AFFECTED ADULT RETINA
Figure 11

Section through the retina of a young adult mouse from the affected stock. No trace of the outer retina remains. Kolmer. Heidenhain's iron haematoxylin. (× 300).

electroretinogram vanishes at a very early age.[7, 11]

In rats and dogs this form of retinal degeneration is often associated with cataract and so is retinitis pigmentosa in man. In the mice described here no cataract was ever seen. In rats suffering from retinal degeneration the cataracts were clearly secondary to the retinal condition since the former never appeared without the latter.[2] On the other hand there must also have been a hereditary factor in the production of this cataract since the incidence of lens opacities was much increased by selective breeding. Since cataract can appear in mice[4] it may well be that a second mutant gene determining its appearance is absent from the present stock.

Summary

1. An hereditary degeneration of the retina in mice is described.

2. The retina develops normally to almost the adult stage when the rods degenerate. Loss of the outer layers of the retina and degeneration of the pigment epithelium follow. There is no secondary cataract.

3. The condition is inherited as a Mendelian recessive character.

4. The relationship of this lesion to retinal degenerations in other animals and to retinitis pigmentosa in man is discussed.

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✦

The Inheritance of Cup Leaf in Cotton

C. F. Lewis*

THE genetic analysis of any species is enhanced by the accumulation of simply inherited characters. Genetically marked stocks have proved to be useful tools in many types of genetic and cytogenetic experiments. Workers with the genus *Gossypium* need a much larger number of genetic mutants for marking chromosomes and establishing more linkage groups.

Some years ago Dr. L. M. Blank (Bureau of Plant Industry, State College, New Mexico) gave the Texas Agricultural Experiment Station a mutant plant which he had found in a field of Stoneville 2B cotton, *Gossypium hirsutum* L. This mutant was designated cup leaf because the adaxial surface curved into a concave form as shown in Figure 12. In addition to the striking cupped appearance of the leaves, the bracteoles were curved slightly and the petals failed to flare open as widely as those of normal flowers. Previous observations, particularly by C. W. Manning (Stoneville Pedigreed Seed Co., Stoneville, Miss.), indicated that the cupped leaf expression was controlled by a single, incompletely dominant gene. The present experiment was conducted in order to obtain critical data on the inheritance of cup leaf and to make them available to other

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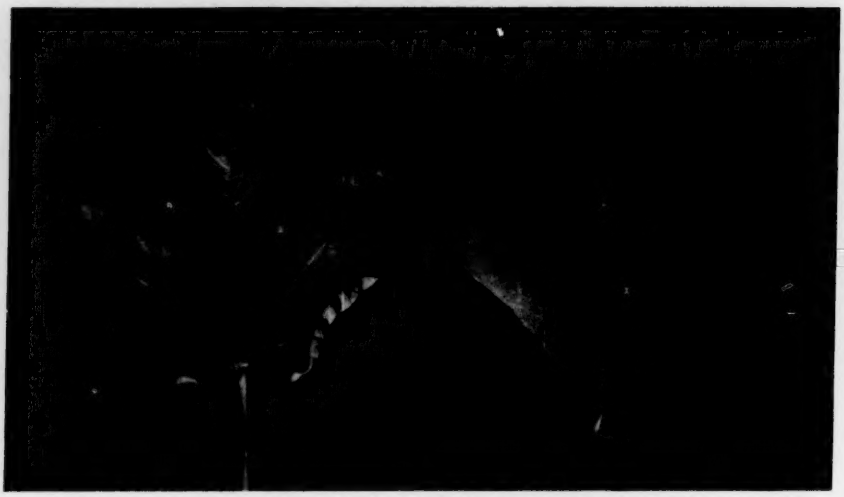

CUP LEAF AND BROAD LEAF COTTON

Figure 12

The cup leaf mutant is shown on the left and normal broad leaf cotton on the right.

workers. The linkage relationship of cup leaf with frego bract, a simple recessive mutant bracteole type described by Green,[1] was also determined.

Procedure and Results

The following three stocks were used in this study: cup leaf—cupped leaves and normal bracteoles; frego bract—broad leaves and frego (narrow and curled) bracteoles; cup-frego—cupped leaves and frego bracteoles. This stock was selected from an F_2 progeny of cup leaf $\times$ frego bract and bred true for both characters the following year.

The F_1 of cup leaf $\times$ frego bract gave an intermediate expression of the cup leaf character. The F_1 was self-pollinated to produce an F_2, backcrossed to cup leaf, and crossed with the tester stock, cup-frego.

In the F_2 the distinction between normal and intermediate cup leaf was more difficult to detect than was the obvious difference between intermediate cup and cup leaf. It was easier to consider cup leaf a recessive and score the progeny into two classes, (1) normal leaf or intermediate cup, and (2) cup leaf. The F_2 segregated into 121 normal or intermediate cup to 41 cup leaf. This does not deviate significantly from an expected 3:1 ratio as indicated by a χ^2 of .008, probability of occurrence .50-.95.

The F_1 backcrossed to cup leaf resulted in a progeny of 73 intermediate cup to 75 cup leaf. Here the χ^2 is .027, probability .50-.95, which demonstrates that a single gene pair is involved in the cup leaf expression.

The test cross of the F_1 $\times$ cup-frego was scored into four phenotypic classes as shown in Table I. The results obtained from this progeny and the analysis of the data are given in Table II.

These results indicate that cup leaf is controlled by a single incompletely dominant gene which is independent of the gene for frego bract.

TABLE I. Phenotypic classes of the F_1 $\times$ cup-frego test cross

Phenotype	No. of Plants
Intermediate cup leaf, normal bracteoles	115
Cup leaf, normal bracteoles	107
Intermediate cup leaf, frego bracteoles	96
Cup leaf, frego bracteoles	101

TABLE II. Analysis of the data resulting from F_1 test cross

Cup leaf vs Intermediate cup leaf	Frego vs Normal bracteoles
208 211	197 222
$\chi^2 = .021$	$\chi^2 = 1.492$
$P = .50 - .95$	$P = .20 - .30$

Linkage

216 203

$\chi^2 = .403$

$P = .50 - .95$

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A GENETIC ANALYSIS OF THE BRITISH TOGGENBURG BREED OF GOATS

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McPHEE and Wright[3] have shown how the genetic history and degree of inbreeding within a breed can be studied by a pedigree-sampling method. Robertson and Asker[5] applied a modification of this method to the study of the British Friesian breed of cattle. This is a large pure breed with a low level of inbreeding. It appeared to be of interest to investigate, by the same method, the genetic history of a small breed recently developed on a crossbred foundation. For this purpose the British Toggenburg breed of goats was chosen.

The aim was to find out whether the small size of this breed had led to a high level of inbreeding within it and, if so, whether there were relatively unrelated strains within the breed so that the actual inbreeding of individual animals could be kept low by crossing these strains.

The herd book of the British Goat Society was first published in 1886 and contained the pedigrees of animals born since 1877. To begin with all animals were listed together, but in Volume II, Part II (published 1906) a Toggenburg Section was started and all animals of pure Toggenburg descent were re-entered in this section. In the 1912 issue of the herd book the Anglo-Nubians were similarly segregated. During the 1920's further divisions were made and the herd book now consists of seven sections. Their names, and the number of animals registered in the 1950 herd book, are as follows:

British Section	737
Anglo-Nubian Section	118
Saanen Section	108
Toggenburg Section	30
British Alpine Section	316
British Saanen Section	587
British Toggenburg	169

The Saanen, like the Toggenburg Section, is restricted to animals of pure Swiss descent. The Anglo-Nubian breed, as indicated by its name, is of crossbred origin. The British Section contains goats of mixed breeding which are eligible for the herd book but not for any of the breed sections. The last three breeds are of recent origin attained by crossing "British" goats with imported Alpine, Saanen or Toggenburg respectively.

The British Toggenburg section of the herd book was opened in 1925 and entry was based on pedigree and type. The conditions of entry have varied in detail from time to time. The current regulations may be summarized as follows: goats may be entered in the British Toggenburg Section if their parents are entered either there or in the Toggenburg Section (or in the short-lived British Toggenburg Register which was a stepping-stone to the Section); they may also be graded-up by three crosses of a registered Toggenburg or British Toggenburg.

The breed has slowly increased in size as shown by the following figures which list the number of registrations in five-year periods since the inception of the breed (Section and Register combined):

1925-29	81
1930-34	101
1935-39	293
1940-44	395
1945-49	810

*Member of Scientific Staff, Agricultural Research Council of Great Britain. The author wishes to thank Dr. Alan Robertson for the assistance and advice given him during the course of this analysis.

BRITISH TOGGENBURG FEMALE

Figure 13

Northmoor Gazelle, a female typical of the breed was born in 1942. She has yielded 31,651 pounds of milk in eight lactations.

On the average, 80 percent of the annual registrations are females. Writing in 1945 Jeffery[2] said there were probably about 750 registered British Toggenburgs in Great Britain and commented that the breed is having difficulty in holding its own in competition with other breeds.

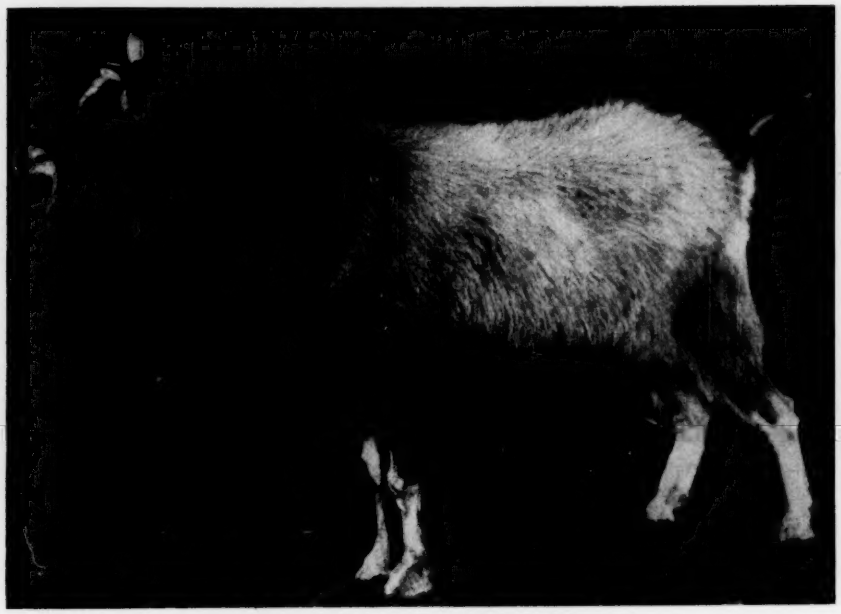
Materials and Methods

A sample of 50 was chosen from the animals registered in the British Toggenburg Section of the 1950 herd book of the British Goat Society. In order to get as broad a sample as possible, only females were chosen and not more than one female was chosen from each herd. The sire and dam of each of these animals were noted, but instead of drawing out the complete pedigree of each sire and dam only two lines were traced back from each. In each generation the toss of a coin decided whether the direct line should pass through the male or the female; the second line behind each sire and dam consisted of the mates of the animals in the direct line. (See Robertson and Mason[6].) Any animal common to the sire's and the dam's pedigree indicates some inbreeding and is called a "tie."

The lines were traced back to Volume II Part II of the herd book which lists Toggenburgs from the beginning and other animals born during 1901-1905 (a few were born during 1898-1900). Some lines naturally stopped sooner by ending in an animal not previously entered in the herd book (either grading-up or imported).

Origins of the Breed

Since we are dealing with a random sample of the present-day breeding stock



TYPICAL MALE BRITISH TOGGENBURG

Figure 14

A male, typical of the British Toggenburg breed of goats is shown. The *inter se* relationship of males and females from the breed is 20.6 percent which is equivalent to an inbreeding of 11.4 percent.

and a random sample of their ancestors, the initial animals in the direct lines will appear in the same proportion, within the limits of error, as if the full pedigrees of all the breeding animals had been drawn out.

In fact, about half the lines began with purebred Toggenburgs and about half with animals of other breeds (see Table I).

Table I shows that at least 47 percent of the genes in the breed derive from imported Toggenburgs. In actual fact the proportion will be higher, since many of the animals labelled "grading-up," i.e. those not previously entered in the herd book, will contain Toggenburg blood. How many and how much, it is impossible to tell from a study of the herd book.

TABLE I. Percentage contributions of different breeds and importations

Breed	Date of Import	Percentage of direct lines		
Toggenburg	1922	17		
	1904	7		
	1896	13		
	earlier	10		
			Total	47
Saanen	1922	3		
	earlier	6	Total	9
Anglo-Nubian				10
Grading-up				34
				100

However, it is possible to determine the date at which "grading-up" occurred. Although 34 lines end in non-pedigree animals, they involve only 17 different animals. Their dates of birth are not known but the dates of birth of their offspring are known (i.e. the offspring which appear in the direct line pedigrees). The distribution of these birth dates in five-year classes is shown below.

1903-07	2
1908-12	4
1913-17	5
1918-22	5
1935	1

Although the numbers are small, it would be safe to conclude that most of the "grading-up," from non-pedigree animals, occurred in the 25 years before the British Toggenburg was recognized as a breed. "Grading-up" from the British to the British Toggenburg Section is still a current practice. Outside blood will also have entered the breed in the Anglo-Nubian. As mentioned above, their pedigrees were not taken further back than 1900 or thereabouts.

Amount of Inbreeding

The extent of inbreeding of the breed in 1950 relative to the year 1901 is obtained by considering the number of "ties," as previously defined, found in the sample pedigrees. Wright has shown that the average amount of inbreeding is given by half the number of animals in the original sample showing ties, divided by the possible number of "ties." In this case two lines were drawn out from the dam and two from the sire of each animal of the sample; there are therefore four possible "ties" per animal. Since there are 50 animals, the possible number of "ties" is 200. The number of "tie" observed was 55. The amount of inbreeding is therefore: $\frac{55}{2 \times 200} = 0.138$ or 13.8 percent. Thus on the average the British Toggenburg breed in 1950 was 13.8 percent more inbred than its ancestors born about the year 1901.

The generation length was roughly estimated by considering the number of generations in the direct line between animals born or imported at a known date. The average generation length between 1900 and 1950 was 4.0 years; between 1922 and 1950 it was 3.5 years. These may seem very long considering that both male and female breed at one year of age. It must be remembered that they represent the average age of animals at the birth of their offspring and popular sires and dams are in use for many years.

An increase in inbreeding of 13.8 percent in 50 years (12½ generations) is 1.1 percent per generation.

A consideration of the animals which form the "ties" shows when the inbreeding occurred. The dates of birth of such animals are distributed as follows (five year classes):

1905-09	3
1910-14	1
1915-19	14
1920-24	4
1925-29	4
1930-34	5
1935-39	12
1940-44	9
1945-49	3

It will be seen that there was a burst of inbreeding in the period immediately before the recognition of the breed and another in the 1930's.

Inter se Relationship

With a given degree of inbreeding all the animals in a breed at any given time may be equally related to each other or they may be grouped into relatively isolated strains within which the animals are more closely related. The extent of this separation into strains may be estimated from a consideration of the collateral or *inter se* relationship of the animals in the breed (r). If there is no separation into strains, this will be double the amount of inbreeding, or more exactly, long-term inbreeding $= \frac{r}{2\text{-}r}$.

The *inter se* relationship of the sires and dams of the sample was calculated. To do this the number of "ties" between each sire and the mate of every other sire was counted. This number was 2018. There are 50 sires and 49 dams (excluding each sire's own mate) and 4 possible "ties" per sire (since there are two lines behind each sire and two behind each dam); thus the total possible number of "ties" is $50 \times 4 \times 49 = 9800$. The relationship between males and females taken at random from the breed is therefore $\frac{2018}{9800} = 0.206$ or 20.6 percent.

This is equivalent to an inbreeding of $\frac{0.206}{2\text{—}0.206} = 0.114$ or 11.4 per cent compared with the actual inbreeding obtained of 13.8 percent. (Using Robertson and Asker's terminology this total inbreeding includes current inbreeding, long-term inbreeding and a factor due to any division into strains. Since the analysis revealed only one "tie" due to current inbreeding in the sample animals—a sire-daughter mating—it was assumed not to be a factor of great importance.)

Discussion

The rate of increase in inbreeding of 1.1 percent per generation may be compared with the figures obtained for various dairy cattle breeds. Robertson and Asker[5] showed that the rate of increase in inbreeding in the British Friesian breed since 1910 has been about 0.16 percent per generation. For the Jersey[7] and the Ayrshire[1] the rates are slightly more, or 0.4-0.5 percent per generation. Considering the small size of their breed, British Toggenburg breeders have been remarkably successful in keeping the inbreeding low. Even if the higher rate of inbreeding since 1915 is considered, the smaller generation lengths mean that the increase is still only 1.5 percent per

generation. This approaches 1.6 percent per generation obtained for the Red Danish breed.[6] However, Robertson[4] has shown that there may be little connection between breed size and "effective number of herds." He shows that although the British Toggenburg has only about 150 annual registrations compared with 28,000 in the Ayrshire, the effective numbers of herds supplying sires, sires of sires, and sires of sires of sires respectively are 122, 25 and 14 in the Ayrshire compared with 18, 13 and 11 in the British Toggenburg. Thus if we go back four generations the two breeds differ very little in effective size.

An examination of the conditions of registration and of the sample pedigrees suggests how the inbreeding has been kept low. At any time purebred Toggenburg blood can be introduced and a relative outcross obtained. The extent of this practice can be judged from the fact that five of the 100 parents of the sample animals were purebred Toggenburgs and likewise 26 of the 200 grandparents. The interesting question arises: what happens in a minute breed like the pure Toggenburg in Britain where inbreeding cannot be prevented by outcrossing?

The difference between the calculated inbreeding of 13.8 percent and 11.4 per cent obtained from the relationship between random males and females might appear to leave room for the development of strains within the breed. An inbreeding of 11.4 percent would correspond to 46 "ties"; in actual fact 55 were found. Since the S.E. for the 46 "ties" is about 3.4 and for the 55 it is about 8.6, the difference between the two estimates is not significant. There is therefore no evidence of the formation of strains within the breed.

Summary

1. By Wright's pedigree sampling method the origin and genetic history of the British Toggenburg breed has been investigated.
2. At least one-half of the genes in the registered females born in 1950 arise from Toggenburgs imported in 1922, 1904, 1896 or earlier. About one-third come from animals graded-up during the period 1903-21, and the rest come from the Saanen and Anglo-Nubian breeds.
3. Relative to animals born about 1901 the breed in 1950 was 13.8 percent inbred. This represents an increase in inbreeding of 1.1 percent per generation.
4. The *inter se* relationship of males and females drawn at random from the breed was 20.6 percent. This is equivalent to an inbreeding of 11.4 percent but the difference is not great enough to warrant the assumption that there are separate strains within the breed.

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(*Received March 16, 1953*)

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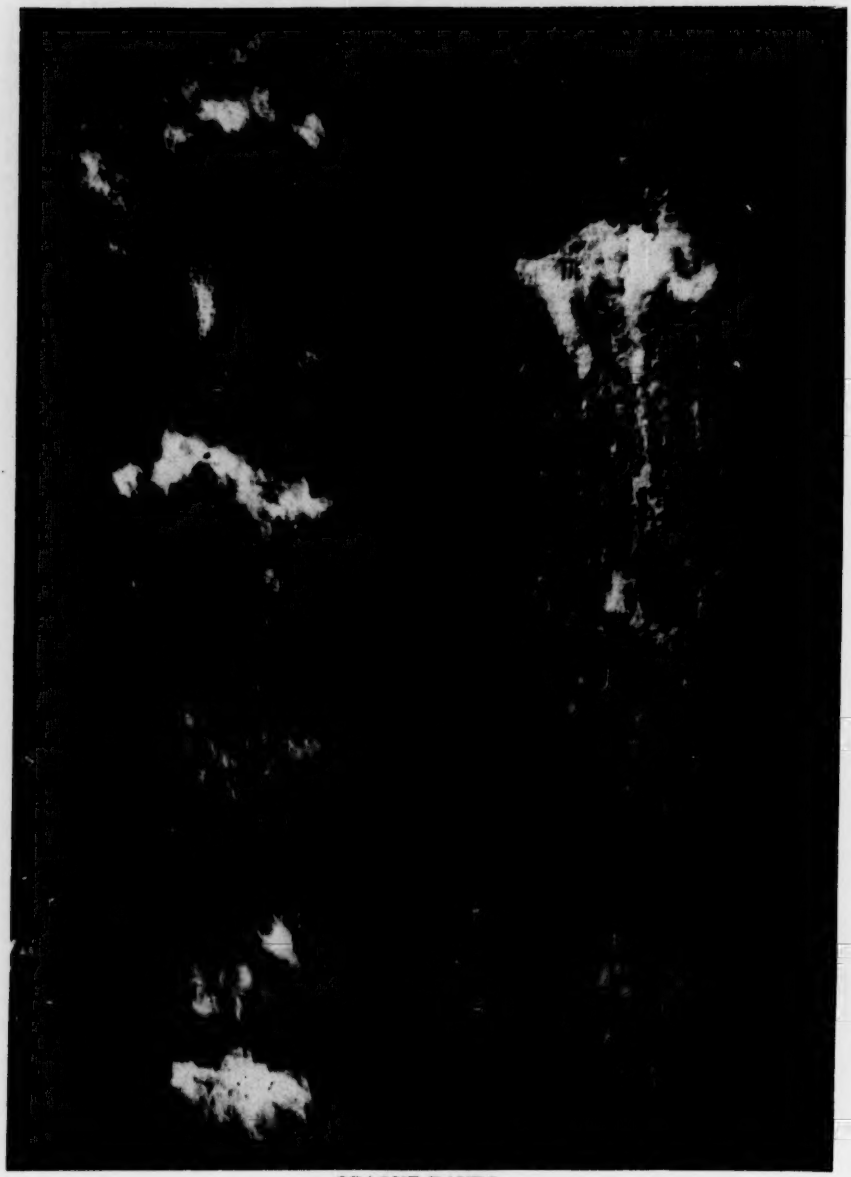
Identical Twins Sought for Study of Multiple Sclerosis

An appeal for identical twins, male or female, in which one or both members suffer from multiple sclerosis, was made recently by the National Multiple Sclerosis Society, in an effort to aid research into the possible hereditary and environmental causes of the crippling disease.

Twins responding to the call will be asked to cooperate in a scientific study of their lives and medical histories. For purposes of the planned research, the ideal twins would be those where one member had developed multiple sclerosis and the other had not. Both would be studied to try to determine which factors may have been responsible for the disease in the afflicted twin.

"Fortunately, nature presents us with a ready-made laboratory experiment in the situation of identical twins," Dr. Harold R. Wainerdi, Medical Director of the Society, states. "Identical twins, having an identical heredity, have facilitated the solution of many other problems in health and disease." (Although the health organization is seeking identical twins for the purpose of its research project, it should also like to hear of all cases of twins afflicted with multiple sclerosis, as well as cases in which the disease has attacked more than one person in a family.)

All persons responding to the Society's appeal for identical twins are asked to contact: The National Multiple Sclerosis Society, 270 Park Avenue, New York 17, N. Y.



OPAQUE BANDS

Figure 15

A—Shows an electron micrograph of a salivary gland chromosome of *Drosophila* larva consisting of regions which are totally opaque to the electron beam. The longitudinally running chromofilaments are variable, according to the type of stretch. *B*—shows one opaque band and two small bands which are less opaque and are composed of fine chromomeres.

ELECTRON MICROSCOPY OF SALIVARY GLAND CHROMOSOMES

G. YASUZUMI AND KEIKO ITO*

IT is most important to clarify the submicroscopic structure of salivary gland chromosomes, because their euchromatic bands have been shown directly or indirectly to contain the genes. Painter[11] has stated that salivary gland chromosomes are multiple threads or of a polytene structure with the qualification that the visible chromomeres and their longitudinal connecting strands are not single gene strings nor simple chromonemata but compounds, in varying degrees, of the two. Bands are made up of chromomeres closely appressed, but a single chromomere must be regarded as compound in the sense that it is made up of a number of homologous ultimate chromomeres, of which none could probably be seen by itself. In other words, the visible single chromomere may be compared to a package of cigarettes and the connecting strands are comparable to cables rather than to single wires. This polytene conception has been supported by electron microscopic observation (Yasuzumi, *et al*[21]).

Yasuzumi and coworkers[16,18] have already demonstrated that the metabolic chromosome isolated from blood cell nuclei of various animals consists of at least eight chromofilaments of approximately 5-10μ in diameter. Ris[14] has also shown that the lampbrush-chromosomes of amphibia are made of submicroscopic fibrils, each forming a small-gyred helix. The present authors propose the expression "chromofilaments" for the threads appearing along the longitudinal axis in the salivary gland chromosome of *Drosophila* according to Yasuzumi and coworkers.

Recently the increased power of resolution of the electron microscope and the progress of technique for specimens have enabled a number of scholars to elucidate the minute structure of salivary gland chromosomes.[1,7,8,12,13,15,17,21,22] The small euchromatic bands are seen to consist of chromomeres, but the structure of the larger ones remains concealed, being opaque to electron beams.

The structure of the lampbrush-chromosome produced by highly alkaline solutions has been observed under the light microscope.[2,9,11] However, the submicroscopic structure of artificial lampbrush-chromosomes has never been observed by an electron microscope.

The present paper is an account of our experiments and conclusions concerning the structural details of euchromatic bands, matrix and lampbrush produced by alkali of salivary gland chromosomes of Diptera. While we believe that these experiments carry our knowledge of the microstructure of salivary gland chromosomes a good deal further, it is clear that much more work remains to be done before a complete picture of the salivary chromosome can be given.

Materials and Methods

Full grown larvae of the wild type *D. melanogaster* and *virilis*, cultured on malt-yeast-agar, have been used for this study. The salivary glands have been placed in a drop of 45 percent acetic acid on a glass slide previously coated with a collodion film approximately 60 mμ thick. In an untreated condition the cell membranes are easily seen, but they are made indistinct by immersion in acetic acid, the nuclei become elongated, and finally the light scattering difference between the nucleus and the cytoplasm disappears under an ordinary light microscope. At this stage, a cover slip is mounted over the material and the preparation is strongly pressed with the finger tip. A piece of blotting paper which is placed upon the cover slip absorbs excess fluid from the preparation. When the preparation dries, it is immersed in 45 percent acetic acid to isolate the cover slip from the slide, leaving the material attached to the collodion film. The collodion film supporting the material is carefully removed from the slide. It is important to select only those slides having

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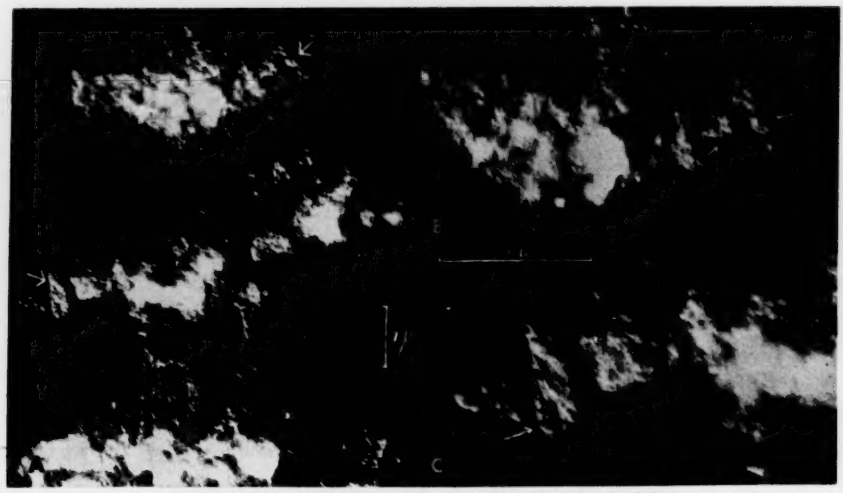
THE CHROMOSOME MATRIX

Figure 16

A—shows the longitudinally running chromofilaments and euchromatic bands enclosed by the matrix, so that their contours appear indistinctly. *B*—shows part of the matrix which has been destroyed so that the chromofilaments can be seen enclosed by the matrix.



CHROMOFILAMENTS AND EUCHROMATIC BANDS

Figure 17

A—shows four bundles each of which consists of longitudinal filaments. An unusually good example of separated chromofilaments and euchromatic bands can be seen. *B* and *C* shows a further enlargement of the upper part of *A*. Part of the dense euchromatic bands observed gives some indication of the presence of helices although these structures appear much more clearly in some bands than in others.

coated collodion films which can easily be removed in water.

For the alkaline treatment the salivary gland is placed in a 1/10 N solution of NaOH. After two to three minutes, the salivary chromosomes are fixed and smeared in 45 percent acetic acid. The specimens are then observed by means of a light microscope to select one ready for electron microscopy. The preparation is shadowed with chromium at an angle of 25°. The typical results obtained by this method using a "Shimazu" SM-1A electron microscope are shown in the accompanying illustrations.

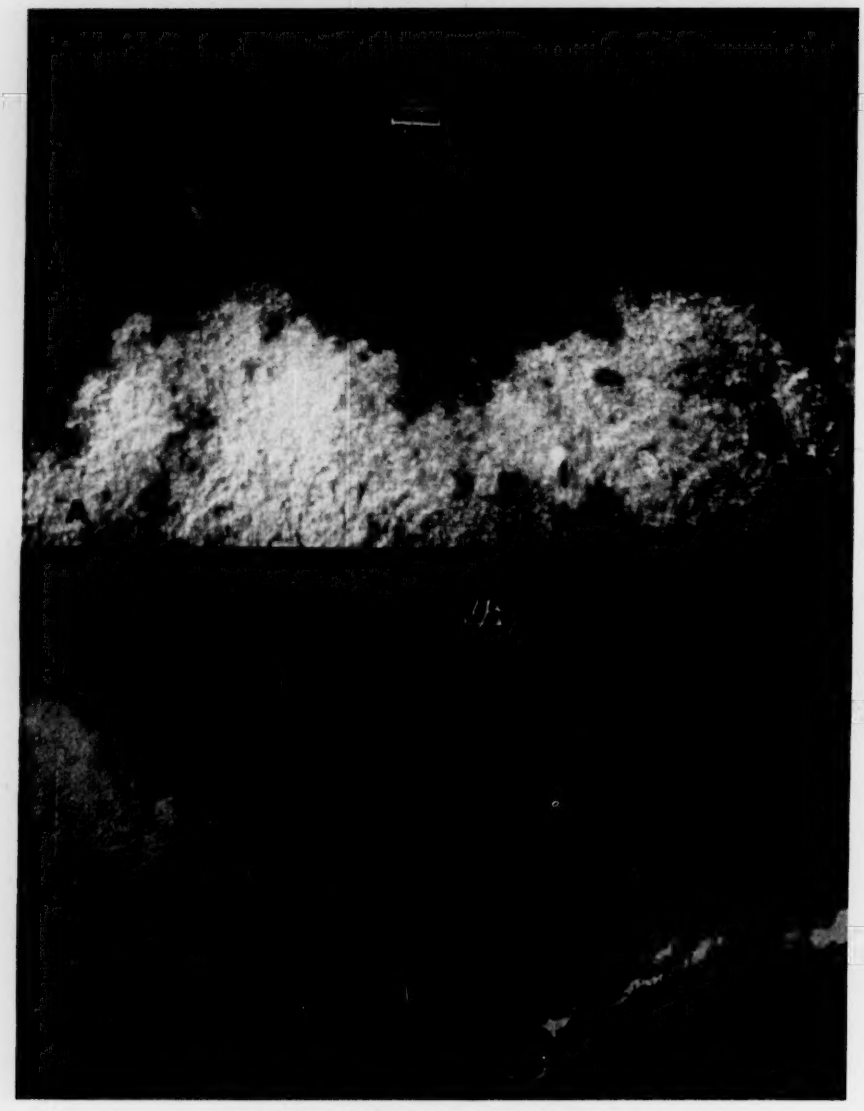
Results

As can be seen in Figure 15*A*, the salivary gland chromosome of the full-grown *Drosophila* larva clearly consists of regions which are totally opaque to the electron beam and a multitude of longitudinally running chromofilaments is variable according to the type of stretch and their width falls between ca. 10 mμ and 30 mμ. Figure 15*B* shows an opaque band and two less opaque small bands which are composed of fine chromomeres ca. 80-160 mμ in diameter. The irregular arrangement of chromomeres is apparently due to the difference in the uncoiling process of individual chromofilaments.

The preparation has successfully been stretched in both longitudinal and transverse axes of the chromosome, so that each interchromatic filament is clearly visible, enabling a part of the dense bands, which have been opaque to electron beams, to be seen clearly (Figure 17). Four bundles, each of which consists of longitudinal filaments are clearly visible in Figure 17*A*. An unusually good example of separated chromofilaments and euchromatic bands is shown in Figure 17*A*. Partial further enlargement is shown in Figure 17*B* and *C*. Part of the dense euchromatic bands observed gives some indication of the presence of helices although these structures appear much more clearly in some bands than in others. Part of the dense band in Figure 17*B* shows a double-stranded helix in a left-handed direction. In Figure 17*C*, a system of minor and major helices can be seen and the constituent filaments of each helix have shown themselves to be helically coiled in a left-handed direction.

Figure 16 demonstrates the presence of a matrix clearly. The longitudinally running chromofilaments and euchromatic bands are enclosed by the matrix, so that their contours appear indistinctly (Figure 16*A*). However, a part of the matrix is satisfactorily destroyed to enable us to observe the chromofilaments enclosed by the matrix (Figure 16*B*).

When the salivary gland is placed in a solution with high pH, the nucleus is swollen and gradually the banded structure of the chromosomes disappears as observed under the light microscope. When the dissolved



ENLARGED LAMPBRUSH-CHROMOSOMES

Figure 18

A and *B* shows electron micrographs of lampbrush-chromosomes. Lateral branches in the images are not the tiny fibers radiating out of the chromosome axis but the reprecipitated substances of dissolved nucleoproteins squeezed out of the swollen and destroyed matrix.

LIGHT MICROGRAPH OF LAMPBRUSH-CHROMOSOMES

Figure 19

A light micrograph of the lampbrush-chromosomes seen in Figure 18. The numerous tiny fibers appear to be radiating out of the individual chromosomes giving a lampbrush effect.

nucleoproteins have not diffused out of the chromosome, most of them are reprecipitated with strong acetic acid. Figure 19 is a light micrograph of lampbrush-chromosomes, and Figure 18 shows electron micrographs of them. In the light microscope figure, the numerous tiny fibers appear to be radiating out of the individual chromosomes, giving a lampbrush image. However, electron microscope observation does not reveal the tiny fibers radiating out of the matrix but it makes clear the reprecipitation of nucleoproteins produced by chemical and physical treatments. The dissolved nucleoproteins are reprecipitated with strong acetic acid and are squeezed out of the swollen and destroyed matrix by the mechanical pressure over the cover slip. The majority of branches projecting laterally from the chromosome axis range from 1.6 to 5.0μ in length.

The matrix is more resistant to the alkali after longer treatment but gradually it swells and allows the electron beam to penetrate. An artificial adhesion of the swollen matrix can often be seen, in which the dissolved nucleoproteins are embedded and are more or less disorganized (Figure 20*A*). The matrix dissolves eventually, and its outline disappears, as seen at the point marked by an arrow in Figure 20*B*. When the matrix has been completely dissolved, most of the nucleoproteins are driven away from the chromosome, and there remains only the reprecipitated part (Figure 21).

Discussion

In order to fully utilize the resolving power of the electron microscope in the study of chromosome structure, only those chromosomes which are thin enough to allow penetration of the electron beam can be used. The squash method is adequate for this purpose, because the whole twisted chromosomes are stretched to come to a clear visualization with no part lost, as Yasuzumi and Odate[20] have described. In the present paper this squash method has provided satisfactory results concerning the structure of euchromatic bands, matrix and artificial lampbrush chromosomes.

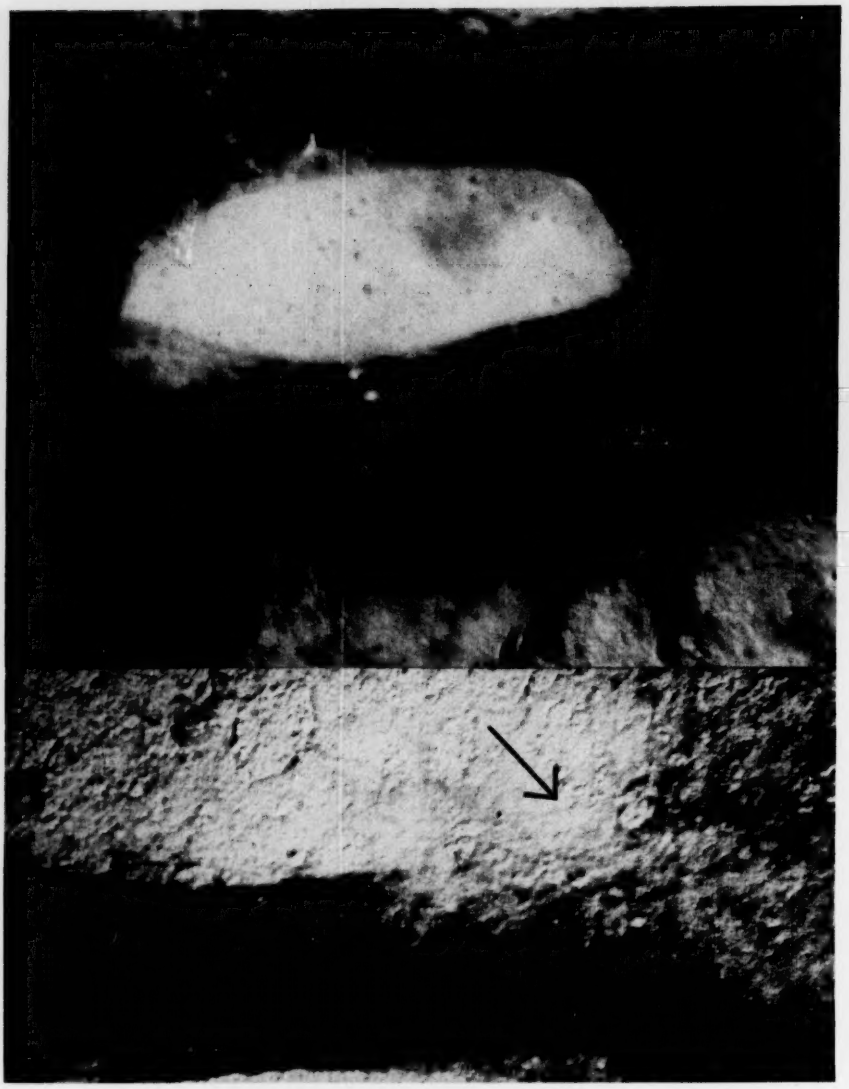
Kodani[9] demonstrated that there were four chromonemata in the salivary gland chromosome. Four bundles, each of which consists of a multitude of longitudinal filaments, have been described in the present research. These bundles seem to correspond to the chromonemata reported by Kodani.[9]

In a previous communication of this laboratory, Yasuzumi, *et al*[21] reported that the longitudinal filaments have a beaded structure with a periodicity falling between ca. 30 mμ and 60 mμ. The present study has shown that the longitudinal chromofilaments are of a spiral nature rather than of a beaded structure and elongated to the width of about 10 mμ. In fact, the appearance of the chromofilaments in Figures 15 and 17 suggests that the extensibility of the chromosome is due to the uncoiling of a spiral of longitudinal chromofilaments.

The present investigation suggests to us that the dense, clear banded structure is composed of a series of coiled Feulgen-positive filaments. A series of these filaments is usually closely appressed, none of which could hitherto be seen singly. This helical structure bears a close resemblance morphologically to the leaf-shaped structure that has been studied by Pease and Baker.[13]

A light microscope observation concerning the process of the transformation of the salivary gland chromosomes to the lampbrush type and other types by treatment with alkali were presented by Calvin, *et al*,[2] Kodani,[9] and Painter.[11] The details of the investigation by an electron microscope presented in this paper are in agreement with the results of Painter, in that the salivary gland chromosome is classified into three types, (see Figures 18, 20 and 21). The explanation which we offer for the sequence of lampbrush-like image formations is as follows: the matrix swells and undergoes disintegration. The dissolved nucleoproteins are embedded in the matrix. The nucleoproteins precipitated with acetic acid are squeezed out of the matrix when the chromosome is pressed transversely to the chromosome axis rather than longitudinally. The lampbrush image has never been observed after treatment with NaOH alone, but it has been revealed after addition of acetic acid followed by physical pressure.

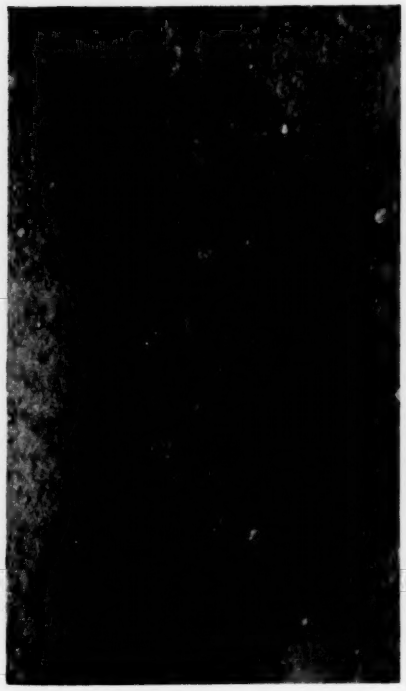
Koltzoff[10] found that the lateral loops attached to the lampbrush-chromosomes of *Triton* and chick are negative to Feulgen's nuclear stain. However, the lateral branches of the artificial lampbrush are Feulgen-positive, and these two structures are not always similar



SWOLLEN AND DISSOLVED MATRIX

Figure 20

A—shows electron micrographs of the matrix of *D. virilis*. Two matrices adhere to each other. *B*—shows a sharp outline of the matrix, but at the right of the figure the matrix has dissolved.



DISORGANIZED NUCLEOPROTEINS

Figure 21

An electron micrograph of the residuum of nucleoproteins after the matrix has been dissolved and most of the nucleoproteins have been driven away from the chromosome of *D. virilis.*

in shape and relative arrangement for the axial strings. Danon,[4] Danon, *et al*,[5] and Clark, *et al*,[3] observed a multitude of loops projecting laterally from the chromosome axis in *Triton* oocytes by means of an electron microscope, and also Gall[6] demonstrated the same structure in the same material by a phase contrast microscope. In view of these results the authors are positive in denying that the natural and experimentally produced lampbrush-chromosomes are identical in structure and chemical components.

In the present studies the matrix has been identified in the chromosome treated with acetic acid alone, and especially when treated with 1/10 N NaOH. It is not certain whether the membrane normally exists before treatment or is a result of treatment; however, the observation of a distinct membrane in the normal chromosome when stained with various glycogen reagents[19] seems to substantiate its existence in the normal chromosome.

Summary

The salivary gland chromosome is composed of a multitude of longitudinal filaments which are of a helical nature rather than of a beaded structure and elongated to the width of ca. 10 mμ.

Electron microscopy furnishes definite evidence for the presence of the matrix in which the structural elements of salivary gland chromosomes are embedded.

The electron microscope images of the artificial lampbrush-chromosomes which are produced with alkali do not explain the structure of the lampbrush-chromosomes found in vertebrate eggs.

The euchromatic band is composed of a series of twisted Feulgen-positive filaments which are coiled in a left-handed direction along the longitudinal axis of the chromosome.

The above mentioned helical structure is visible when the salivary chromosome is successfully stretched to both longitudinal and transverse axes of the chromosome and the matrix is dissolved away from the chromosome.

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HEREDITARY FEMALE STERILITY IN HOLSTEIN-FRIESIAN CATTLE

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GREGORY *et al.*,[2] presented evidence of a specific sex-limited gene (or genes) conditioning female sterility in Jersey and Holstein-Friesian cattle. They defined as fertile any cow that had given birth to a calf carried to normal term; and as sterile any heifer, free of infectious disease, that failed to produce a calf after repeated services to one or more known fertile bulls. These definitions disregard the quantitative aspects of sterility. The report of Fincher and Williams,[1] of a Holstein-Friesian bull that produced sterile daughters from sire-daughter matings was cited. A genetic analysis of this data indicated that an autosomal sex-limited recessive gene conditioned the sterility. It remains a moot question whether the sterility studied by Gregory *et al.*, and by Fincher and Williams is conditioned by the same gene.

Gregory *et al.*,[2] and Mead *et al.*,[4] presented evidence indicating that different genes were probably involved in conditioning sterility in the Holsteins and Jerseys. In a later paper, Gregory *et al.*,[3] presented additional genetic data on the Jersey type of female sterility.

The purpose of this report is to present further evidence of the Holstein type of female sterility.

Source of Data

The data were obtained from the Holstein-Friesian herd maintained by the Newlands Field Station at Fallon. The herd was established in 1925 from a mixed grade and purebred foundation. The majority of the 41 foundation cows were obtained from one breeder, and were linebred to a prominent Holstein bull. (Since this bull was not owned by the University of Nevada and he is the foundation sire of a large line of contemporary Holsteins, his identity is not divulged.)

Every sire used during the succeeding seven generations involved in this study was descended from this bull. No females were purchased during this interval, and considerable genetic assortative mating was practiced. Sire-daughter matings were frequent.

Throughout most of the period of this study the herd served primarily as a source of ani-

(*Continued on page 145*)

INCREASE IN FREQUENCY OF A DELETERIOUS RECESSIVE GENE IN MICE

From a Population Under Selection

E. J. WARWICK AND W. L. LEWIS*

LUSH[4] and others have pointed out that a preference for individuals heterozygous for deleterious recessive genes may be responsible for keeping the frequency of such genes at higher levels in a population than would be expected from the intense selection practiced against the gene through discarding all individuals homozygous for it.

The apparent increase in recent years of a recessive type of hereditary dwarfism in several breeds of beef cattle has led to the conjecture that breeders may be selecting for the heterozygotes. Attempts to identify the possible characteristics of heterozygotes which would cause them to be favored in selection have as yet proved fruitless (Lush and Hazel,[5] Gregory, *et. al.*[1]). The purpose of the present observation is to describe a case in the house mouse where selection apparently greatly increased the frequency of a deleterious recessive gene which produces complete sterility in individuals homozygous for it. Selection against the homozygotes is thus automatically 100 percent. To the knowledge of the authors, this is the first time a case of this kind has been demonstrated experimentally.

In 1948 a selection experiment with the house mouse was initiated at Purdue University based on stock acquired from Dr. J. W. MacArthur of Toronto University. Details of this experiment are given in another paper (Lewis and Warwick[3]). Crosses were made between a stock which Dr. MacArthur had selected for small body size for 25 generations and a stock of medium size which had been allowed to intermate at random for about the same period of time. Selection was then practiced for small 60-day weight for five generations among the resulting progeny.

Selection was practiced in four inbred lines (each composed of one or two males and 10 females per generation) and an outbred population of a size equal to the total of the inbreds and descending from common foundation stock.

No known hereditary abnormalities were detected in the foundation stocks nor in the original crosses. In the first generation of selection one dwarf or "pygmy" individual was noted among 504 individuals raised to 21 days of age. The frequency of pygmies increased in the ensuing generations (see Table I). As will be seen from this table, the proportion of pygmies increased until in generations three to five inclusive, 15.5 percent of all individuals born in lines or breeding groups producing pygmies were of the pygmy type. Three out of four inbred lines produced pygmies as did the outbred population. One inbred line apparently did not contain the pygmy gene as no pygmies were ever produced in the line.

So far as was known at the initiation of the experiment, the foundation stocks were free of any recessive genes for dwarfism. However, King[2] reported that dwarfism had appeared among the progeny of some mice of the small line he acquired from Dr. MacArthur in 1948. He termed this condition "pygmy" and presented data indicating that it was inherited as a simple recessive. The dwarfs appearing in our experiment were phenotypically similar to those he reported and were readily distinguished from normals by their small size, short ears, and tendency toward priapism in the males. It, therefore, seems highly probable that the pygmy gene was present in very low frequency in the small stock originally acquired. When the pygmies began appearing, a decision was made to ignore them in selection; that is, no attempt was made to select against litter mates or other relatives of dwarfs if they were of small size and were otherwise eligible for use as breeding animals. The pygmies themselves proved to be 100 percent sterile so they were not selected as breeding animals.

The most logical explanation for the increase in frequency of this condition in our lines is that the gene for pygmy is not completely recessive. The heterozygotes are probably on the average enough smaller than homozygous normal individuals to be favored in selection. It was naturally possible to practice more intense selection among males than females. A higher percentage of selected males was

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proved heterozygous than was the case with females even when allowance is made for failure to detect heterozygous females. This adds weight to the assumption that selection favored heterozygotes. From the figures it appears that the frequency of the gene for pygmy climbed from a negligible level to over .30 by the third generation and thereafter remained about this level. If this represents the true situation, it would correspond to a case where a heterozygote had approximately twice the probability of leaving offspring as a homozygous normal individual.

In addition to illustrating that selection for a desired character can increase the frequency of a deleterious recessive gene, our data also add to King's evidence that the pygmy condition is due to the action of a single pair of genes.

As shown in Table I, approximately 31.4 percent of the individuals born in litters with one or more pygmies were pygmies. Since the females in this experiment produced only one litter each, it was not possible to calculate the frequency of pygmies in later litters of animals proved heterozygous by their first litter. It would be expected in litters averaging 5.35 (as was the approximate size in the later generations of this experiment) that approximately 21.5 percent of the matings of heterozygous × heterozygous individuals would produce all normal offspring. On this assumption, the calculation of the probable number of heterozygous females producing no pygmies is given in Table II. Adding the number of individuals they would have produced on the average to the normals produced in litters having at least one pygmy, 24.3 percent of the offspring from probable heterozygous × heterozygous matings were pygmies. This is close enough to the expected 25 percent to lend additional weight to the hypothesis that pygmies are the result of the homozygous condition of a single gene.

Summary

The frequency of a presumably recessive gene for a pygmy condition increased greatly during five generations of selection for small size in a mouse population composed of both inbred and outbred segments. It appeared that the heterozygous individuals were enough smaller on the average than homozygous normals to have approximately twice the chance of being selected for breeding purposes and producing offspring.

TABLE I. Occurrence of pygmies in a population of house mice selected for small 60-day weight

Generation of Selection	All Mice Alive at 21 Days of Age			Males		Females		All Mice Alive at 21 Days of Age in Litters with at Least One Pygmy		
	No. Pygmies	No. Normal	Percent Pygmies*	No. Siring Litters	No. Siring Pygmies	No. Producing Litters	No. Producing Pygmies	No. Pygmies	No. Normal	Percent Pygmies
1st	1	503	.2	16	1	71	1	1	3	25
2nd	36	365	9	11	7	69	21	36	82	30
3rd	46	331	12	13	7	68	28	46	108	30
4th	73	303	19	10	10	70	41	73	143	34
5th	46	268	15	16	13	64	33	46	110	29
Totals Gen. 1-5	202	1770	10	66	38	342	124	202	446	31
Totals Gen. 3-5	165	902	15	39	30	202	102	165	361	31

*This population was divided into inbred and outbred segments and was definitely not mating at random. However, so far as is known, within each breeding group the mating was at random with respect to the gene for pygmy. Thus, the frequency of the gene for pygmy can be estimated according to the Hardy-Weinberg formula as the square root of the proportion of pygmies. These estimates are .004; .300; .347; .436; and .388, for generations 1 to 5 inclusive.

TABLE II. Calculations on frequency of pygmies in litters produced by probable heterozygote × heterozygote matings

Generation of Selection	No. Females Proven Heterozygous	No. Additional Females Probably Heterozygous*	All Mice in Litters with at Least One Pygmy		Assumed Nos. Normal Mice in Litters of Females Probably Heterozygous but Producing no Pygmies
			No. Pygmies	No. Normals	
1st	1	?	1	3	—
2nd	21	6	36	82	32
3rd	28	8	46	108	43
4th	41	11	73	143	59
5th	33	9	46	110	48
Totals	124	34	202†	446†	182†

*Based on proportions of heterozygote × heterozygote matings which would on the average fail to produce any pygmies in litters averaging 5.35 mice. $(3/4)^{5.35} = .215$.

†Probable proportion of pygmies in litters from heterozygote × heterozygote matings equals $(202)/(202+446+182)=.243$.

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Hereditary Female Sterility in Holstein-Friesian Cattle

(*Continued from page* 142)

mals for nutrition and management studies. These studies were of such nature as to preclude them as an environmental cause of the observed sterility. There have been few reactors to the test for brucellosis, and these were immediately removed. Trichomoniosis, vibrio fetus, or other specific infectious agents that reduce fertility were not diagnosed or suspected in the herd during the period of this study.

Analysis of Data

Seven generations of females beyond the foundation herd were involved in this study, which covered approximately 25 years. Only a few males were raised. A total of 31 sterile females, as defined by Gregory *et al.*,[2] were included. A veterinarian's examination was not available for all sterile animals. It is possible therefore, that some of the sterility was due to environmental causes.

Except for three heifers born in the first generation each sterile female could be traced through sire and dam to the original foundation bull. Pedigrees of the dams of the three first generation females were not available. However, they were obtained from a herd that was line bred to the foundation bull, so that it is quite likely they were related. The data support the hypothesis of Gregory *et al.*, that an autosomal recessive gene conditions the sterility. Since most bull calves were not used as sires, the data provide no information concerning the sex-limited nature of the gene. However, the Holstein bull reported by Gregory *et al.*, was a son of the first sire used in this herd and a grandson of the foundation bull. It seems reasonable, therefore, to conclude that the same gene is involved in both herds. These data were not sufficiently extensive to provide a reliable estimate of the frequency of the gene, and test Mendelian ratios.

In addition to the 31 sterile heifers there were six that produced one calf, but were incapable of reproducing thereafter. They also traced through sire and dam to the foundation bull. Gregory *et al.*,[3] similarly described several Jersey heifers. In these heifers meiotic activity appeared normal and it was found that ova were capable of fertilization and cleavage. It seemed likely that some substance or reaction necessary for implantation of the foetus may be lacking or blocked or that endocrine dysfunction may be involved; but the sterility might be temporarily overcome, permitting a normal gestation.

These data suggest that those females which produced only one calf might be genetically sterile. Incomplete penetrance or variable expressivity would describe, but not account for, such gene action. A knowledge of the physiological basis of the sterility is required for a complete understanding of the problem.

Summary

An analysis of female sterility, occurring in the University of Nevada Newlands Field Station Holstein-Friesian herd is presented. The data support the hypothesis of Gregory *et al.*, that female sterility is conditioned by a single sex-limited autosomal recessive gene. It is suggested that cows which produce only one calf might be genetically sterile.

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COLOR GENOTYPES OF THE GREENHOUSE SNAPDRAGON

Antirrhinum majus L.

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IN 1907, Wheldale[2] illustrated snapdragon phenotypes in color, and gave F_1, F_2, and F_3 data to show color inheritance. Since that time inbred snapdragon strains adapted to greenhouse culture have been widely adopted by the florist trade. Seikel and Geissman[1] have established color genotypes for two of the varieties listed. A continued interest in F_1 material and the lack of genetic information on commercial strains makes a review of color inheritance desirable at this time.

Materials and Methods

A total of 60 varieties from commercial sources were each inbred one or more generations, then used as parents of F_1's. As soon as different genotypes were identified, all possible combinations were made. For clarity this report is limited to 11 varieties differing genotypically, and to segregations involving all four of the color factors described.

Following accepted practice, each color factor is identified by the first letter or syllable of the adjective describing the recessive phenotype.

Results

The color phenotypes of 54 F_1 populations are given in Table I. The simplest satisfactory hypothesis to explain these results requires the use of four color factors indicated for each variety by symbols at the right of the table.

White (*w*) is homozygous recessive in varieties 1, 2, 3, and 4. The same factor limits pigment formation in each of these varieties because all of the crosses between them yielded uniformly white progenies. Furthermore it is evident that all *ww* individuals will be white regardless of the genetic composition with respect to the other color factors.

Yellow (*y*) is homozygous recessive in varieties 1, 5, 6, 8, and 10. This factor is expressed only in the presence of dominant white. The dominant allele produces ivory base pigmentation, the recessive, yellow. Neither *Y* nor *y* interferes with the expression of either of the following factors.

Ivory (*iv*), like white, inhibits anthocyanin formation in homozygous recessive individuals. Phenotypically it is readily distinguishable from recessive white. Recessive genotypes are represented by varieties 1, 2, 5, 6, and 7.

Dilution (*dil*) is expressed only in individuals showing anthocyanin pigmentation. The recessive reduces purple to pink, and red to bronze.

The above genetic hypothesis may be converted to an F_2 expectation (Table II) based on the monohybrid 3:1 segregation. The numeral 1 indicates the need of the homozygous recessive, 3 the dominant, and 4 either, for the production of the phenotype in question. The product of each line is the expectation for the phenotype. The sum of expectations must equal 4 raised to the power of the number of segregating factors. This system is recommended for the teaching of multiple factor inheritance.

Variety 3, Armstrong's White $\times$ 6, Ball Gold, yielded a uniform purple F_1 progeny. A progeny of 178 F_2 individuals yielded color segregation shown in Table III. The *yy*:*Y*- segregation 27·109 observed differs from the expectation of 34:102 by an amount approaching significance indicating a possible

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lack of viability of *y* gametes or *yy* zygotes. Other mono- and di-hybrid ratios conform closely to expectation.

Variety 2, Peace, × 10, Scarlett O'Hara, gave a purple F_1 which yielded an F_2 shown in Table IV. Again the *yy*:*Y*- segregation of 13:60 differed from the expectation of 15.25:54.75 by an amount approaching significance, $\chi^2 = 2.038$, P = .1, indicating a doubtful deficiency of the homozygous recessive genotype. Other mono- and di-hybrid ratios conform to expectation.

The purple F_1 of variety 11, Windmiller's Lilac × 1, Yuletide, was backcrossed to the recessive parent, Yuletide, yielding a backcross progeny shown in Table V. In this segregation the recessive ivory individuals in the yellow and ivory classes totaled 59 which exceeds the expected 47.25 to give a chi square value of 2.92 and a probability between .05 and .1 indicating some possibility of recessive advantage.

The recessive dilution individuals from the pink and bronze classes total

TABLE I. Variety and F_1 Colors

	Yuletide	Peace	Armstrong's White	Rockwood's Crystal White	Ethel	Ball Gold	Margaret	Lady Dorothy	Cheviot Maid	Scarlett O'Hara	Windmiller's Lilac	Genotype
1. Yuletide	White	White	White	White	Yellow	Yellow	Ivory	Bronze	Pink	Red	Purple	*ww yy iviv dildil*
2. Peace		White	White	White	Ivory	Ivory	Ivory	Pink	Pink	Purple	Purple	*ww YY iviv dildil*
3. Armstrong's White			White	White	Pink	Purple	Pink	Pink	Pink	Purple	Purple	*ww YY IvIv dildil*
4. Rockwood's Crystal White				White	Purple	Purple	Purple	Purple	Purple	Purple		*ww YY IvIv DilDil*
5. Ethel					Yellow	Yellow	Ivory	Bronze	Pink	Red	Purple	*WW yy iviv dildil*
6. Ball Gold						Yellow	Ivory	Red	Purple	Red	Purple	*WW yy iviv DilDil*
7. Margaret							Ivory	Pink	Pink	Purple	Purple	*WW YY iviv dildil*
8. Lady Dorothy								Bronze	Pink	Red	Purple	*WW yy IvIv dildil*
9. Cheviot Maid									Pink	Purple	Purple	*WW YY IvIv dildil*
10. Scarlett O'Hara										Red	Purple	*WW yy IvIv DilDil*
11. Windmiller's Lilac											Lavender	*WW YY IvIv DilDil*

TABLE II. Phenotype Expectation

Phenotypes	Genes: White	Yellow	Ivory	Dilution	Product Expectation
White	1	4	4	4	64
Yellow	3	1	1	4	12
Ivory	3	3	1	4	36
Bronze	3	1	3	1	9
Pink	3	3	3	1	27
Red	3	1	3	3	27
Purple	3	3	3	3	81
Total	4	4	4	4	256

TABLE III. F_2 Four Factor Segregation of Armstrong's White × Ball Gold

Phenotype	Genotype	Observed	Expected
White	*ww - - -*	42	43.5
Yellow	*W- yy iv iv -*	7	8.3
Ivory	*W- Y- iv iv -*	28	25.4
Bronze	*W- yy Iv- dil dil*	4	6.2
Pink	*W- Y- Iv - dil dil*	24	19.0
Red	*W- yy IV - Dil -*	16	19.0
Purple	*W- Y- Iv - Dil -*	57	56.4
Total		178	

$\chi^2 = 3.1004$ P = .7-.8

TABLE IV. F_2 Four Factor Segregation of Variety Peace × Scarlett O'Hara

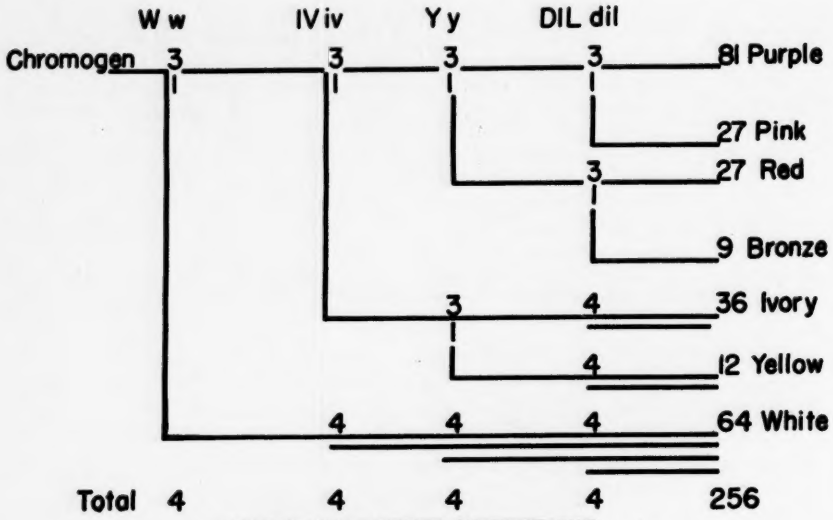
	Observed	Expected
White	23	24.0
Yellow	5	4.5
Ivory	16	13.5
Bronze	2	3.36
Pink	8	10.1
Red	6	10.1
Purple	36	30.4
Total	96	

$\chi^2 = 3.3223$ P = .7

TABLE V. Backcross Four Factor Segregation

	Observed	Expected
White	96	94.5
Yellow	29	23.625
Ivory	30	23.625
Bronze	7	11.8125
Pink	7	11.8125
Red	6	11.8125
Purple	14	11.8125
Total	189	

$\chi^2 = 10.664$ P <.1



SNAPDRAGON COLOR INHERITANCE

Figure 22

Multiple lines indicate epistasis and the numerals give F_2 expectations for the segregation at each locus. Product of numerals between chromogen and phenotype on each path give F_2 phenotype expectations as in Table II.

only 14. The expectation for this group was 23.625. Chi square value is 3.9, probability between .02 and .05, indicating some heterozygote advantage. This segregation gives no evidence of linkage.

Discussion

The epistatic relations of these factors suggest different ontogenetic sequence of their actions. Assuming that the several colors originate from a common precursor or chromogen, the process of formation of dominant purple color may be interrupted at different points of development by lack of dominant color factors yielding the seven observed phenotypic classes as illustrated diagrammatically in Figure 22.

TABLE VI. Gene Designations

Phenotype	Wheldale	Geissman	Haney
White	*yy — — ··*		*ww -- — ··*
Yellow	*Y - ii rr —*	*-- yy pp MM**	*W - yy iv iv —*
Ivory	*Y - I - rr —*		*W - Y - iv iv —*
Bronze	*Y - ii R - bb*		*W - yy Iv - dil dil*
Pink	*Y - I - R - bb*	*-- YY PP mm†*	*W - Y - Iv - dil dil*
Red	*Y - ii R - B -*		*W - yy - Iv - Dil -*
Magenta	*Y - I - R - B -*		*W - Y - Iv - Dil -*

* Ball Gold † Cheviot Maid 33

Conclusion and Summary

The results obtained in this study conform so closely to those published 46 years ago by Wheldale[2] that there can be little doubt that the same color factors are involved. Identification of the other 49 known variety genotypes will be published in the Bulletin of the National Snapdragon Society.

Eleven of a possible 16 genotypes for four color factors established by parent and F_1 colors and confirmed by F_2 and backcross segregations re-establish the validity of Wheldale's[2] 1907 analysis of color inheritance in the snapdragon.

Genotypes available in commercial trade are identified for genetic experiment or demonstration.

A simple method of determining phenotypic expectation from known gene interactions in multiple factor inheritance is demonstrated.

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2. WHELDALE, M. The inheritance of flower colour in *Antirrhinum majus*. *Proc. Roy. Soc.* Lond. LXXIX B: 288. 1907.

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